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THE IMPACT OF MALARIA ON LIPIDS AND GLUCOCORTICOIDS IN CHILDREN UNDER TEN YEARS AT THE KORLE BU TEACHING HOSPITAL

BY

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DECLARATION

I, Benedict Nii Ayi Armah, do hereby declare that the work presented in this dissertation was carried out by me at the Department of Chemical Pathology, University of Ghana, Legon, under the supervision of Prof. Henry Asare-Anane and Dr. Seth. D. Amanquah.

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DEDICATION

This work is dedicated to my mother Emelia Lartey and my uncle Mr. Solomon Lartey.

ACKNOWLEDGEMENT

My deepest gratitude and thanks goes to Emelia Lartey and Solomon Lartey for their support, I would like to express my sincere gratitude to my supervisors Professor Henry Asare- Anane and Dr. Seth. D. Amanquah for their assistance in painstakingly making time to assist and advise me throughout my project work. Your efforts have indeed helped me put up this great master piece, I am forever grateful.

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LIST OF ABBREVIATIONS

AChE	Acetyl Cholinesterase
ACTH	Adrenocorticotrophin Hormone
ADP	Adenosine Diphosphate
B_0	Maximum Binding
CBG	Cortisol Binding Globulin
CHD	Coronary Heart Disease
CRH	Corticotrophin Releasing Hormone
ELISA	Enzyme-linked Immunosorbent Assay
GCSH	Glucocorticoid Steroid Hormones
HDL-Chol	High Density Lipoprotein Cholesterol
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
K_2EDTA	Di-potassium Ethylenediaminetetracetic Acid
NSB	Non-Specific Binding
pH	Power of Hydrogen
PRR	Pattern Recognition Receptors
TA	Total Activity

TCA Cycle	Tricarboxylic Acid Cycle
TG	Triglycerides
$TGF_{\beta}1$	Transforming Growth Factor Beta 1
TNF	Tumor Necrosis Factor
Total-Chol	Total-Cholesterol
WHO	World Health Organization

ABSTRACT

Background: Malaria is a common and life-threatening disease in Ghana. Malaria infection has been implicated in lipid and glucocorticoid imbalances among children. Cortisol-induced stresses and parasitaemia may affect brain development and risk of cardiovascular disorders among children.

Aim: To investigate the impact malaria has on lipids and glucocorticoids in children.

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Method: A comparative cross-sectional study using random sampling method was used in this study conducted between the month of February and May, 2019. A sample size of 77 participants comprising 46 cases and 31 controls were involved in the study. Thick and thin blood smears were made for each participant, stained with Giemsa and examined under microscope. Plasma total cholesterol, triglycerides, HDL and LDL were estimated using a chemistry analyzer. Cortisol levels of participants were measured by Enzyme-linked immunosorbent assay.

Result: *Plasmodium falciparum* was responsible for all identified cases of malaria infection in this study. The prevalence of malaria in this study was 59.7%, ages 1-5 years (n=11) had a prevalence of 23.9% whiles 6-9 years (n=35) had a prevalence of 76.1%. Children aged 6-9 years were two times more likely to get malaria than those in the 1-5 years group (OR=1.966, $p<0.001$). HDL-Chol associated negatively with level of parasitaemia (rho=-0.538, $p<0.0001$). Triglycerides correlated weakly but positively with malaria count (rho=0.296, $p<0.05$). No association were observed for LDL-Chol, VLDL-

Chol and Total-Chol versus malaria count respectively ($p > 0.05$). Cortisol was not associated with level of parasitaemia in this study ($p > 0.05$).

Conclusion: This study showed no association between cortisol and malaria infection among subjects. HDL-Chol impacted negatively with level of parasitaemia. The implications of malaria on glucocorticoids however merit further research.

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria is a common and life-threatening disease in several tropical and subtropical sectors with highest prevalence in Africa (WHO, 2012). Malaria has been found in over 100 countries and over 125 million international travellers visit these countries every year (WHO, 2012). The burden of malaria is heaviest in Africa, where an estimated 91% of all malaria deaths occur in children (WHO, 2018).

The cause of malaria infection was discovered more than a hundred years ago. Malaria still remains one of the contributing factors of mortality and morbidity in the tropical sector or area (Province, 2003). The worldwide prevalence of malaria is estimated to be 300 - 500 million clinical cases and over 1.5 million deaths occur mostly in children per annum, which represents 3% of the overall global disease burden (Province, 2003).

In certain epidemiological situations, malaria can be a devastating disease with high mortality and morbidity that deserves rapid response (Control, Mass, & Movements, 2003). In other locality, while malaria may not be an important cause of severe illness and death, it may have a more pressing public health impact through increased morbidity, loss of productivity, and aggravation of other problems, such as anemia and malnutrition in children (Control, Mass, & Movements, 2003).

Funding for malaria control and eradication campaigns has leveled off since 2010. In 2016, over 2.7 billion dollars (US$) was invested in malaria outreach programmes globally (WHO, 2018). The 2.7 billion (US$) represents less than half (41%) of the

estimated 6.5 billion (US$) needed yearly by 2020 in order to reach the global malaria eradication target in 2030 (WHO, 2018).

Malaria incidence is common among the poorest areas of the society, due to individual's inability to afford safety from malaria through better-quality housing, indoor residual spraying, anti-malaria drugs and clean environment (Landau, 2012). Individuals are also vulnerable because of ineffective treatment and diagnosis of malaria leading to fatal consequences (Landau, 2012).

Glucocorticoid steroid hormones are affected by stress such as generated by malaria infection (Raffington, 2018). A study by Raffington, (2018) found that the developing brain of children are vulnerable to the effects of stress which was determined by elevated levels of glucocorticoid hormones. Furthermore patients with high parasitaemia of *P. falciparum* were shown to have high levels of cortisol (Jacob, 2016). Patients with malarial infection show a wide range of metabolic derangements including changes in serum lipid profile and corticosteroids, these changes in serum lipid profile and their possible correlation with malarial infection (Warjri et al., 2016).

Malaria is widespread and persistent in all parts of Ghana, There are seasonal variations that are evident in the northern part and southern part of the country (Ghana Malaria Operational Plan, 2018).Seasonal variations affects the span of malaria transmission which differs by the geographic area in Ghana, during the span of the dry season (December to February) there is little transmission (Ghana Malaria Operational Plan, 2018). During the span of the raining season (July to November) there is high transmission (Ghana Malaria Operational Plan, 2018).

Ghana's population is estimated at 29 million with a high risk of malaria infection (Ghana Malaria Operational Plan, 2018). Children and pregnant women are at higher risk of severe illness due to complicated and naive immunity respectively (Ghana Malaria Operational Plan, 2018). *P. falciparum* accounts for 85-90% of all malaria infections with *P. malariae* also accounting for less than 10% and more rarely *P. ovale* accounts for 0.15% (Ghana Malaria Operational Plan, 2018). Cases of *P. vivax* infection has however not been detected in Ghana (Ghana Malaria Operational Plan, 2018).

1.2 Problem Statement

Malaria kills a minimum of three (3) children daily and is the highest among OPD cases in Ghana (G.H.S., 2017). Malaria places a burden on the government and individuals through treatment and control measures whereby millions of cedi's are invested in malaria programmes (G.H.S., 2017). Plasmodium can divert and salvage cholesterol from the host as it replicates inside the liver cells (Warjri *et al*., 2016). In order to maintain viability the parasite has to import these nutrients from the host, causing derangements in lipids (Warjri *et al*., 2016). This leads to low level of cholesterol thereby affecting the production of the glucocorticoid hormones, which consequently inhibits gluconeogenesis, suppress immune responses and inflammation (Addison, 2012). Reduction in glucocorticoid hormones production due to malaria infection can affect normal homeostasis and might lead to the severity of the infection.

Children under the age of ten years are mostly affected by cortisol imbalances as a result of malaria infection, these leads to a high morbidity and mortality (Lamb, 2012).

1.3 Justification

In spite of the accessibility of malaria control measures and intervention, the morbidity and mortality is still high in Africa (WHO, 2018). In Ghana cases of inadequate treatment of malaria is quite high among the urban and rural areas (Diallo, 2016). Africa continues to bear 90% of malaria cases and 91% of malaria deaths worldwide (WHO, 2018). Little information has been documented on glucocorticoids imbalances by malaria infection in children less than ten years, A study by Raffington, (2018) found that the developing brain of children are vulnerable to the effects of stress which was determined by elevated levels of glucocorticoid hormones.

There is thus, an urgent need to investigate the impact that malaria have on lipids and glucocorticoids among these children that are undergoing rapid brain and physical development. It is also hoped that the findings generated from this study will make several contributions to both knowledge and understanding of malaria, lipids and glucocorticoid function. Findings from this study could inform the Health Sector on the clinical relevance of glucocorticoids in the treatment and holistic management of malaria in Ghana.

1.4 Null Hypothesis

Malaria infection has no effect on glucocorticoids and lipids.

1.5 Aim:

To investigate the impact malaria has on lipids and glucocorticoids among children under ten years.

1.6 Specific Objectives:

1. To determine the levels of Lipids (Total-Chol, HDL-Chol, LDL-Chol and TG) in malaria infected and control subjects to indicate the contributing effect of malaria on the various lipids.

2. To determine the levels of cortisol in malaria and non-malaria patients to see the effect of parasitaemia on the stress hormone.

3. To determine the association between cortisol and malaria in subjects

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to malaria

Global increase of malaria is focused in the tropics and subtropics around the equator, because of sufficient rainfall, warm temperatures and stagnant water that provide ultimate habitats for Anopheles mosquito larvae (WHO, 2012). World Health Organization (WHO) considers malaria to be endemic in over one hundred and six (106) countries worldwide, with about ten (10) countries in the eliminating phase (WHO, 2012).

Malaria transmission is by an infected female Anopheles mosquito making it a vector borne infectious disease. Malaria infection is caused by protozoan parasites (ANON, 2013). The cause for malaria was basically unknown until the late 19th Century, the primitive Italian term ‘mala -aria’, meaning "bad air" (ANON, 2013).

There are five known species of malaria parasites that are capable of infecting humans, *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* (CDC, 2018). *P. knowlesi* is mainly known to induce malaria infection in monkeys and it is able to infect humans with malaria (CDC, 2018). The most virulent species are *P. falciparum* and *P. vivax*, In 2011 they were responsible for an estimated 660 000 deaths (WHO, 2012). Whilst both *P. ovale* and *P. malariae* are known to cause less life threatening diseases, an estimated 200 million reported cases of malaria in 2011 had been contributed by all these parasites (WHO, 2012).

Malaria infection can be transmitted from a mother to her foetus before or during delivery because the malaria parasites are present in erythrocytes and malaria transmission can be

through the shared use of needles, organ transplant, syringes contaminated with blood and blood transfusion (Bakheet & Alla, 2015).

2.1.1 Malaria Life Cycle

The life cycle of malaria starts when an infected female anopheles mosquito bites and introduces sporozoites into the blood of its host (Ridley, 2012). As soon as the sporozoites get in the liver, they slide along the sinusoidal epithelium crossing numerous kupffer cells (where the liver macrophages resides) and then invade the final hepatocyte (Bakheet & Alla, 2015). The sporozoites undergo cytoplasmic division which is also known as schizogony and numerous nuclear division (Greenwood, 2012). The schizont in the infected hepatocyte develops into a large exo-erythrocytic or liver-stage form, which yields between 10,000 and 20,000 merozoites over a five to ten days (5-10) time period (Greenwood, 2012).

The life cycle of malaria parasites in the liver stage allows the parasite to multiply and it is not linked with any symptoms or disease in malaria infection (Lamb, 2012). Recurrent malaria infections occur due to dormant liver-stage parasites known as hypnozoites and is caused by *P. ovale* and *P. vivax*, the hypnozoites are mostly resistant to anti-malarial drugs (Lamb, 2012). The merozoites then erupts from the final hepatocyte and then undergo asexual erythrocytic cycle after invading the red blood cells of the host, replicating parasites digest haemoglobin to obtain the amino acids they require for their viability (Bakheet & Alla, 2015). The malaria parasite ingests 75% of the host erythrocytes (Red blood cells), the parasite breaks down the haemoglobin into iron and globin chains (Van Heerden, 2002). The iron is used by the parasite for haem synthesis and electron transfer (Van Heerden, 2002). The free haem (Fe^{2+}) ferrous-protoporphyrin

IX produced after the degradation of the haemoglobin can become toxic to the parasite because it lacks haem oxygenase activity, the parasite converts the free haem into a less toxic form called hemozoin (Van Heerden, 2002). The free haem (Fe^{2+}) ferrous-protoporphyrin IX undergoes oxidation to form (Fe^{3+}) ferric-protoporphyrin IX (haemetin) (Van Heerden, 2002). The haemetin precipitates to form hemozoin under acidic conditions. If the process of forming the hemozoin is delayed or inhibited, the reactive oxygen species may induce DNA damage, oxidative stress, lipid peroxidation of parasite membranes and parasite death (Van Heerden, 2002).

About 10-32 merozoites are released when the infected red blood cells erupts during schizogony and these merozoites invade fresh red blood cells to begin a new erythrocytic cycle (Bakheet & Alla, 2015). A small proportion of infected red blood cells differentiate into male and female gametocytes, the exact processes which steers the molecular growth of female and male gametocytes are not known (Bakheet & Alla, 2015).

The zygotes develop into ookinetes, the ookinetes traverse the extracellular space between the midgut tissues and overlying basal lamina and then develop into oocysts (Bakheet & Alla, 2015). The oocysts burst open after nine to twelve days, where about thousands of sporozoites are been released (Bakheet & Alla, 2015). Once the sporozoites are released they travel through the haemolymph and they become attached to the basal lamina of the mosquito salivary glands, the mosquitoes transfer the sporozoites into the next host (Bakheet & Alla, 2015).

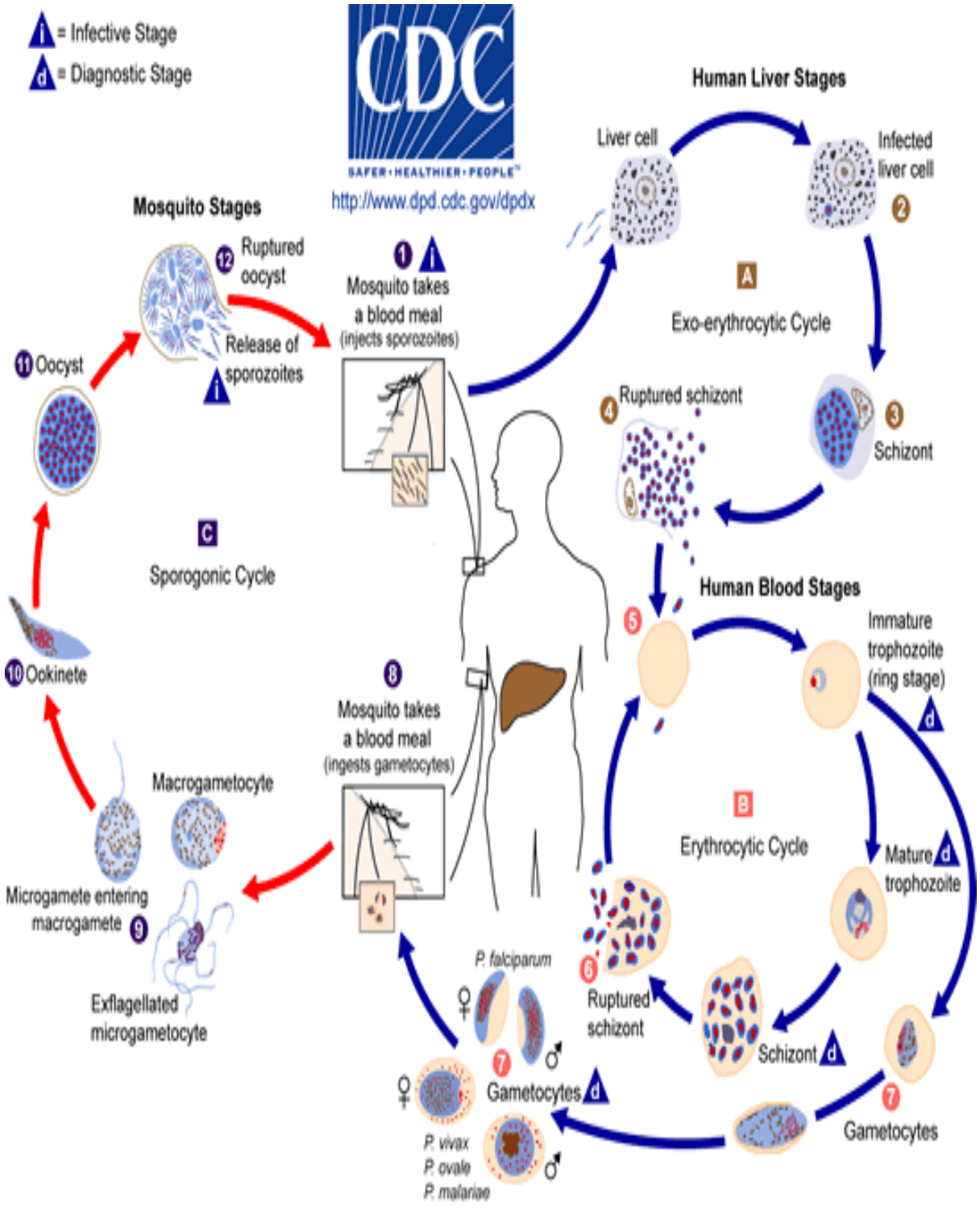


Figure 1 Malaria Life Cycle of *Plasmodium Falciparum* (CDC, 2018).

2.1.2 Complications in Malaria

Malaria infection leads to development of metabolic acidosis which occurs when there is loss of bicarbonate from the body by the kidneys or the pH of the blood is lowered due to increased production of hydrogen ions resulting in respiratory distress (Lamb, 2012). The intensity of malaria infection is fatal and can lead to high morbidity and mortality rate (Lamb, 2012). In severe malaria infection, the volume of circulatory blood decreases leading to severe anaemia which worsens the metabolic acidosis state of the patient (Lamb, 2012). Chronic and recurrent infections of malaria in many cases is associated with severe anaemia with a fall blood level to <5 g/dl (Range: 10-15 g/dl for humans) (Lamb, 2012).

The immune system removes infected red blood cells from circulation as part of it immune mechanism clearance of foreign bodies. Immune responses to parasite products such as haemozoin lowers the normal hematopoietic mechanisms in the spleen and bone marrow (Bakheet & Alla, 2015).

Hypoglycaemia is often associated with malaria complications, the complications leads to loss of appetite (starvation) which contributes to reduction in glucose levels, consumption of glucose by parasite and impairment of gluconeogenesis by cytokines (Ogetii *et al*., 2010).

Cerebral malaria causes brain injury which shows a long-lasting neurocognitive impairments in certain patients with active treatment , it also has a high morbidity and mortality rate (Bangirana *et al*., 2016). Cerebral malaria is characterized by coma, impaired consciousness and blood smears of malaria parasites (asexual forms) (Bangirana *et al*., 2016).

2.1.3 Plasmodium Falciparum Virulence

The *Plasmodium specie* is known for causing severe malaria disease, *P. falciparum* differs from the other *plasmodium species* and its virulent to humans in various aspects (Ribacke, 2009). Examples of the special features are the ability to invade erythrocytes of all ages, multiply asexually at high rates and evade the host immune system through sequestration and antigenic variation (Ribacke, 2009). All these special features result in high parasite loads in the host, which has been shown to correlate to severity of the disease and also the ability to sequester which is considered being the major virulence trait of *P. falciparum* (Ribacke, 2009). Sequestration causes obstruction of blood flow which could lead to hypoxia and release of inflammatory mediators, thus resulting in raised intracranial pressure (Ribacke, 2009) .

Normal erythrocytes change form upon infection by *P. falciparum* when the severity is gradually increased as the parasite matures in the erythrocyte, Normal erythrocytes display a remarkable deformability (Ribacke, 2009). Erythrocytes changes form slightly when infected by a ring-stage trophozoite, the erythrocyte turn impossible to deform when infected with mature trophozoites or schizont stages and also the severity has been suggested a result of membrane modifications by parasite proteins (Ribacke, 2009).

2.2 Strategies to Eliminate and Control Malaria

The use of insecticide treated mosquito nets (ITNs), suppression of erythrocytic-stage malaria infection in human (chemoprevention), rapid diagnosis and treatment of malaria infection (case management) and indoor residual spraying (IRS) reduces vector transmission of parasites from humans to mosquitoes and then back to humans (figure 4).

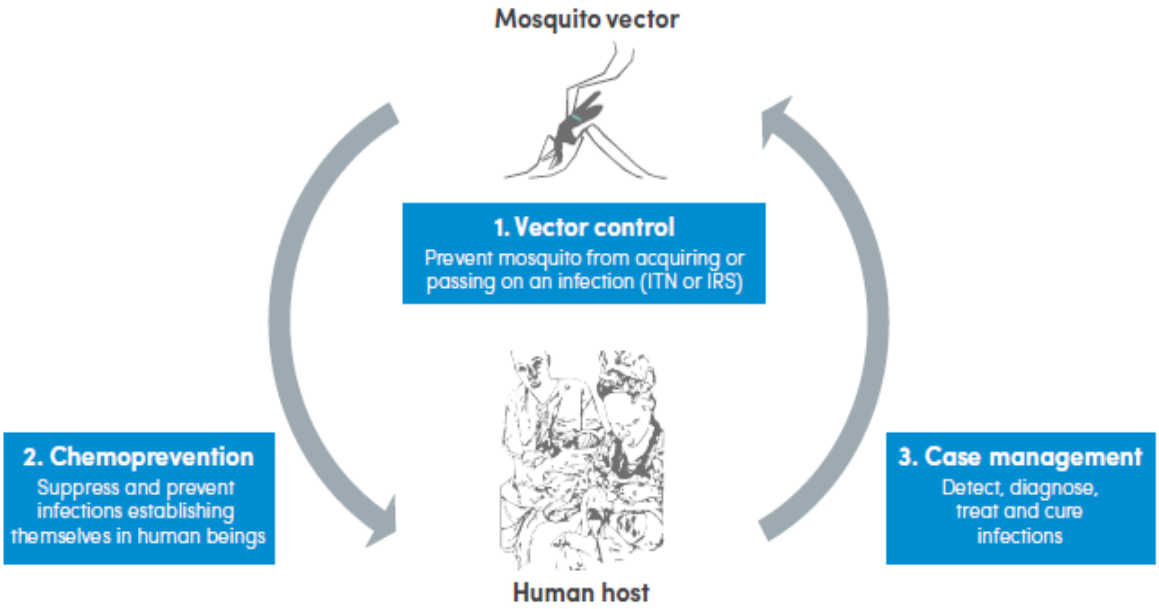


Figure 2 – Strategies to prevent malaria (Diallo, 2016)

Malaria mortality and morbidity rate in children in Africa has been reduced with the use of insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) by an estimated 55% (Diallo, 2016). The impact of insecticide-treated mosquito nets (ITNs), anti-malaria drugs and indoor residual spraying (IRS) have been beneficial to health sector due to reduction of malaria deaths and also reductions in child deaths from other causes that are associated with malaria (e.g. acute respiratory infection, low birth weight and malnutrition) (Diallo, 2016).

2.3 Treatment

Malaria is a preventable and treatable disease, the main objective of treatment is to ensure a complete and rapid elimination of the parasite from the blood in order to prevent development of uncomplicated malaria to cause severity of the disease, chronic infection

and rapid replication of parasites can lead to malaria-associated anaemia (Bakheet & Alla, 2015). Treatment of malaria infection by intravenous infusion can be administered to patients who cannot take oral anti-malarial drugs or severe malaria (Bakheet & Alla, 2015). The medications used in treatment of malaria include: Artemether-lumefantrine, atovaquone-proguanil, mefloquine, quinine, quinidine, artesunate, doxycycline (used in combination with quinine), clindamycin (used in combination with quinine) and these drugs are mostly active against the plasmodium species in the blood (Bakheet & Alla, 2015). In addition, primaquine is active against hypnozoites (dormant parasite liver forms) and it also prevents recurrent malaria infection (Bakheet & Alla, 2015). Primaquine should not be prescribed for pregnant women and individuals who are deficient in glucose-6- phosphate dehydrogenase (G6PD). Patients who have been excluded of G6PD deficiency from the screening test should only take primaquine (Bakheet & Alla, 2015).

Children with malaria infection who are deficient in Vitamin A should be provided with Vitamin A tablets or by infusion, Thus Vitamin A plays a vital role in the human body by promoting proliferation and differentiation of T cells, Good vision and Hematopoiesis (Sanjoaquin & Molyneux, 2009).

2.4.1 Lipid Disorder

Malaria has effects on the lipids and leads to lipid abnormalities; Low levels of total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins(LDL) accompanied by high levels of triglycerides and very low-density lipoproteins (VLDL)

characterize the lipid changes in malaria (Luiz, Vieira, Gonzalo, & Rivera, 2017). Such defects occur in the most prevalent species of plasmodium as well as in complicated and non-complicated cases (Luiz *et al*., 2017). Lipid deposits are important vitality stores for some life forms including man, lipoproteins are made up of particles consisting of apoproteins and core lipids (Ogbodo, 2008). Lipoproteins are responsible for transportation of triglycerides and cholesterol in circulation (Ogbodo, 2008).

Lipoproteins consist of high density lipoprotein (HDL), chylomicrons, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) (Ogbodo, 2008). Several disease conditions including hypertension, diabetes, liver disease and atherosclerosis have been associated with increased level of total cholesterol, increased level of low density lipoprotein (LDL) and low or moderate level of high density lipoprotein (HDL) as a result of defects in the metabolism of lipids and lipoprotein (Ogbodo, 2008).

In malaria, oxidation of lipoproteins takes place in the liver. The oxidation process modifies the lipoproteins and the degree of modification is linked to the severity of the malaria infection (Ogbodo, 2008). Consequently, the oxidation of lipoprotein enhances the cytoadherence of the infected red blood cells (Ogbodo, 2008). Malaria parasites increases its pathogenicity by taking advantage of the oxidative stress (Ogbodo, 2008). For instance, the oxidation of low density lipoprotein increases the endothelial expression of adhesive molecules in malaria patients and this suggests the increase pathogenicity of the disease by oxidized lipoprotein (Ogbodo, 2008).

Malaria parasites do not have any biological pathway for sterol synthesis for their metabolic requirements, the parasites obtains cholesterol from the liver of the human host for their growth and development (Luiz *et al*., 2017).

2.4.2 Cortisol

Cortisol is an important glucocorticoid produced from cholesterol in the adrenal cortex (figure 3). The corticotrophin-releasing hormone (CRH) from the hypothalamus which stimulates the release of Adrenocorticotrophin hormone (ACTH) and is inhibited by the negative feedback of cortisol (Addison, 2012). About 75% of cortisol is protein-bound in circulation, it is bound to transcortin which is known as the cortisol binding globulin (CBG) (Addison, 2012). Cortisol increases hepatic gluconeogenesis during fasting and the release of Acetyl CoA from the muscle serves a substrate for the TCA cycle which is required for gluconeogenesis ,it increases the release of free fatty acid and glycerol by lipolysis (Addison, 2012). Cortisol inhibits glucose uptake in the adipose tissues and muscles. The anti-inflammatory actions of cortisol inhibits lymphocyte production and also decreases the migration of inflammatory cells to the sites of injury (Addison, 2012). Activation of hypothalamus/pituitary/adrenal axis occurs due to the release of cytokines or as a result of stress generated by malaria infection, the basal and peak levels of Adrenocorticotropic hormone is associated with cytokines level (Ibrahim *et al*., 2011). Glucocorticoid steroid hormones (GCSH) are affected by stress exposure. The developing brain of children are more vulnerable to the effects of stress than the adult brain and also animal models have also presented patterns of elevated glucocorticoid hormones depending on the exposure of stress (Raffington, 2018). The level of cortisol upon awakening in the first hour follows a diurnal variation with increased levels of cortisol and a decreased level of cortisol throughout the rest of the day (Raffington, 2018). A study by Jacob, (2016), revealed that patients with high parasitaemia had high cortisol than those with low parasitaemia,and he also revealed that malondialdehyde and

blood glucose were higher in malaria patients than the control group. A study by Tayeb,(2015) revealed that patients with high parasitaemia had high levels of cortisol level as compared to the control group.

2.4.3 Aldosterone

Aldosterone is a mineralocorticoid hormone and it is secreted by the adrenal cortex, aldosterone is vital to life because it regulates potassium, sodium and chloride (Yan & Hongbao, 2009). Aldosterone induces the excretion of potassium and reabsorption of sodium in the kidney consequently controlling the body's water and electrolyte balances (figure 2) (Yan & Hongbao, 2009). Electrolyte imbalances are common clinical manifestations in several infectious diseases including malaria; Hyponatraemia, Hyperkalaemia, Bicarbonate loss develops because of infection with Plasmodium. Electrolyte imbalance appears because of malaria and may lead to the severity of the disease (Rani *et al*., 2015).

Acid-Base disorders are important because of mortality in severe malaria (Das, 2014). Severe malaria can lead to acute renal failure which is associated with both hyperkalaemia and acidosis (Das, 2014). Symptoms of adrenal gland dysfunction include; fatigue, weight changes, vomiting, nausea, high blood pressure (Yan & Hongbao, 2009). Aldosterone has a clinical evidence on its effect on the heart and kidney with its association on blood pressure (White, 2003).

2.5 Immunity to Malaria

2.5.1 Innate Immunity

Innate immunity refers to central, non-immune mechanisms of host defense against malaria (Bakheet & Alla, 2015). *P. falciparum* infects both old and young red blood cells while *P. vivax* and *P. ovale* infect only young erythrocyte, whereas *P. malariae* only infects old erythrocyte, this occurs due to age of the red blood cells (Bakheet & Alla, 2015). Effector genes are initiated by the activation of macrophages through 'toll-like receptors' in mammals; the products of the effector gene controls and executes the innate defense in a large form of parasitic, bacterial and viral systems (Perlmann, 2002) .

2.5.2 Adaptive Immunity

Acquired or adaptive immunity in malaria stimulates both humoral and cellular immunity by invading parasites. In adaptive immunity also all immunoglobulin's against malaria parasites in the liver, sexual and asexual blood stages develop in malaria patients (Bakheet & Alla, 2015).

The proteins of immune defense which includes; Immunoglobulin M (IgM), Immunoglobulin G (IgG) and Immunoglobulin A (IgA) against asexual blood stages may possibly protect the individual by inhibiting erythrocytes invasion and antibodies against sexual stages thus reduce the transmission of malaria (Bakheet & Alla, 2015).

Depending on the type of interaction between the parasite and host cells leads to the production of cytokines, where the pattern-recognition receptors (PRRs) which are located on host cells can be used to recognize and respond to plasmodium species (Langhorne, 2008). Compromised immune systems of pregnant women and naive

immune systems of children makes them vulnerable to this disease and so are considered to be the highest risk populations for malaria-related deaths (Abossie, 2017).

2.5.3 The Immune System and Cortisol

The immune system has an influence on the endocrine system, cytokines regulate and interact steroidogenesis which affects the functioning and development of adrenal gland (Rohleder, 2002). Synthesis of cortisol by the endocrine cells is been affected by certain cytokines such as TGFβ1 and TNF (Kamal, 2009). The endocrine system also has influence on the immune system, cortisol inhibits cytokines production (Kamal, 2009). Cortisol suppresses the immune system which leads to a decrease in lymphocyte production, decreasing phagocytosis of infected cells, increasing blood sugar and blood pressure (Kamal, 2009).

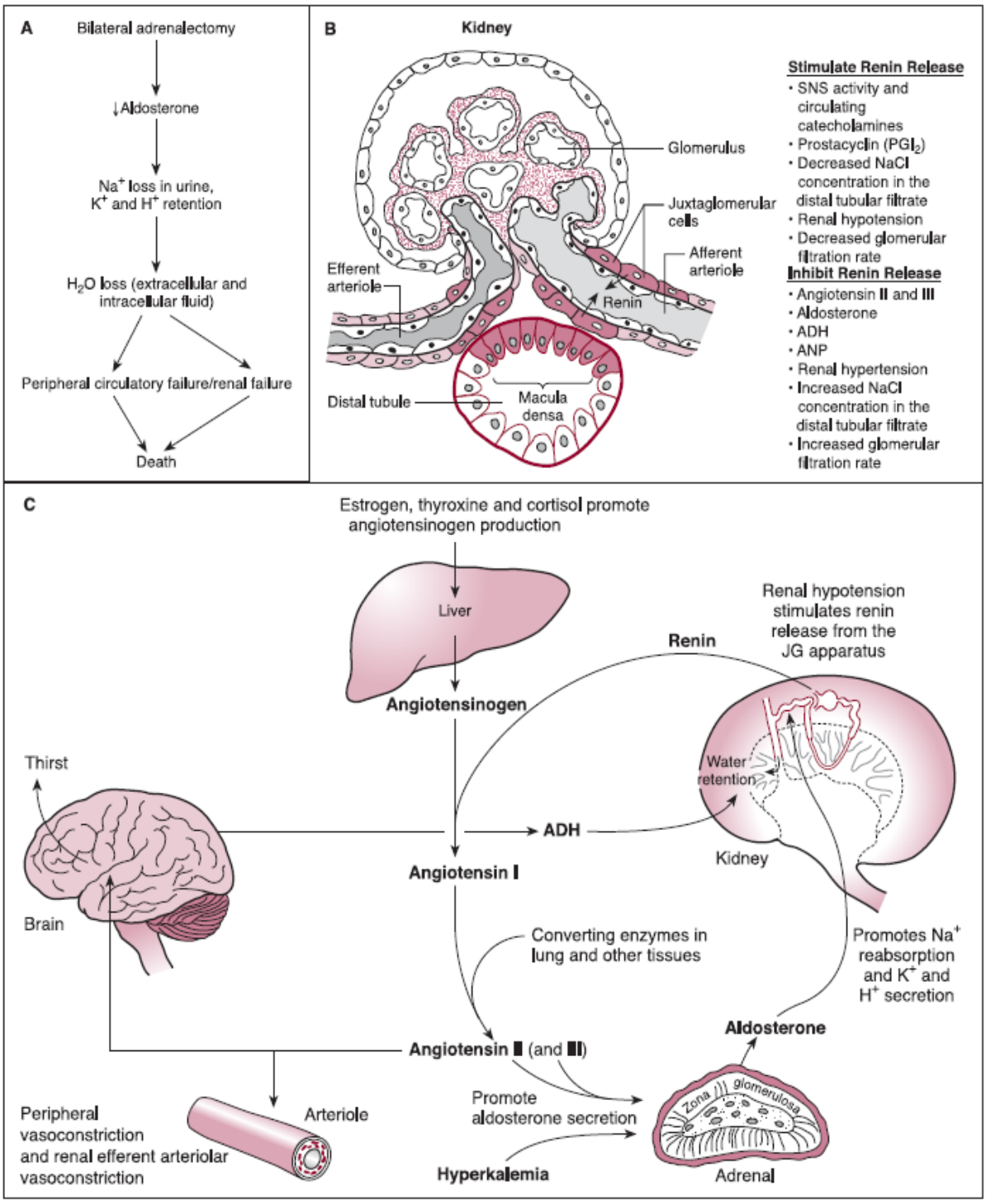
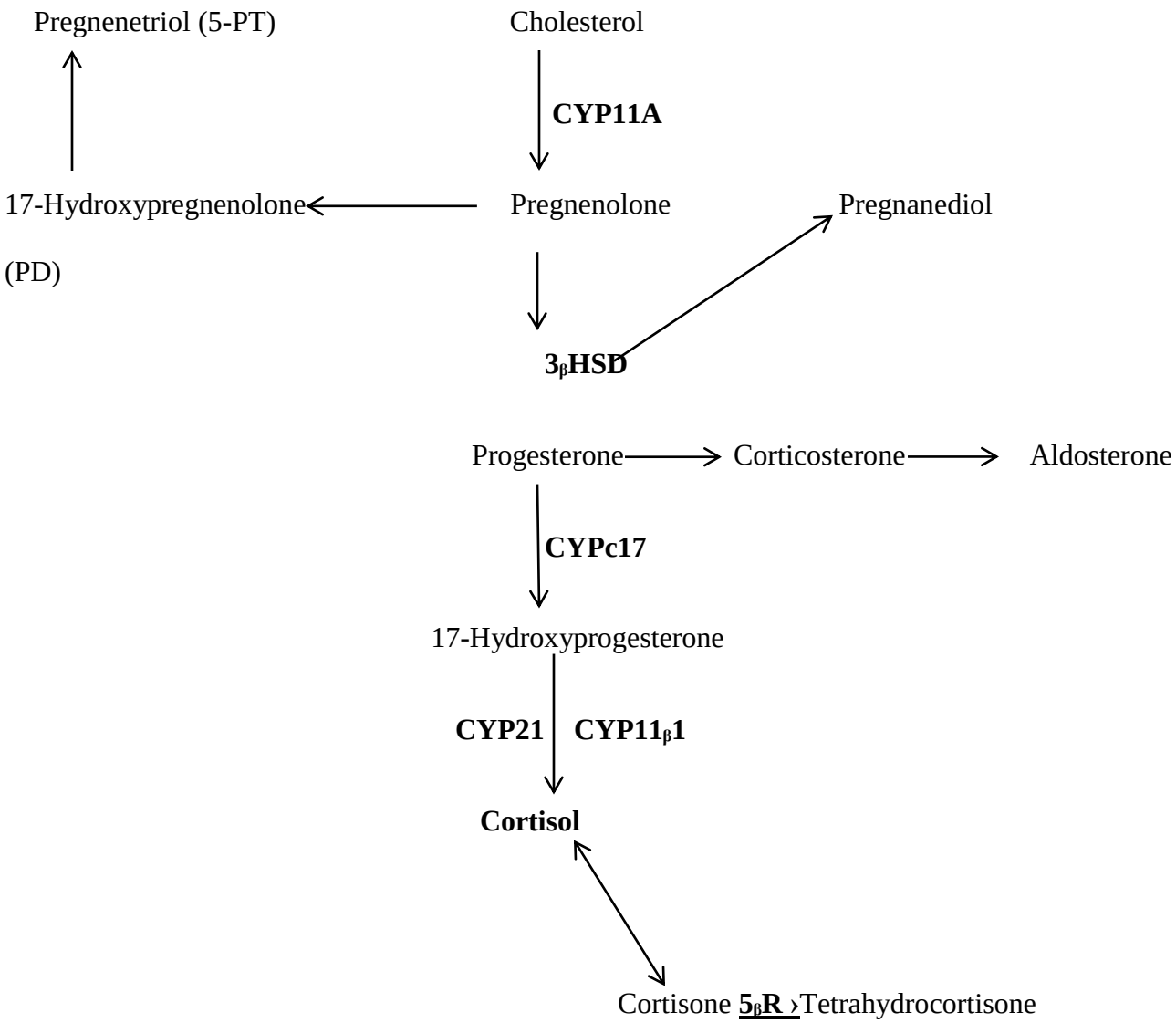


Figure 3: The renin-angiotensin system

(JB Lippincott,Ganong WF, 2017)



Key Enzymes

CYP21: 21-Hydroxylase

5βR: 5-beta-Reductase

3βHSD: 3-beta-Hydroxysteroid dehydrogenase

CYPc17: 17-alpha-Hydroxylase

CYP11β1: 11-beta-Hydroxylase

Figure 4: Biosynthesis and Metabolism of Cortisol (Nordt, 2007)

CHAPTER THREE

METHOD AND MATERIALS

3.1 Study design:

Comparative Cross-sectional study design

3.2 Study Site:

The study was conducted at Korle Bu Teaching Hospital. The Hospital lies between longitude 0° 13' 23.20" East and latitude 5° 32' 9.71" North in the Greater Accra region. It is the centre of excellence for healthcare services, a premier teaching hospital for University of Ghana (College of Health Sciences) and numerous health institutions in the Greater Accra Region of Ghana. It serves as the leading national referral center in the country. The Central Laboratory Service of the Korle-Bu Teaching Hospital, together with its satellite laboratories forms the Lab SUB BMC. As part of its role as a center of excellence in laboratory diagnosis, the Central Laboratories Quality Assurance office actively participates in quality standard assessment towards international accreditation by the International Accreditation Organization (ISO15189) and African Society for Laboratory Medicine (ASLM). When this research is complete, a copy of the report will be made available to the Laboratory Quality Assurance Department. Korle Bu and its immediate environ municipalities have a high record for malaria cases and has a constant annual incidence of cases above 100/1,000 inhabitants.

3.3 Sample Size

The sample size was calculated from the formula below,

$N= 2 (z_\alpha + z_\beta)^2 / \Delta^2$

For $\alpha = 0.05$, $z_\alpha = 1.96$; for $\beta = 0.20$, $z_\beta = 0.84$. Hence $2 (z_\alpha + z_\beta)^2 = 2(1.96 + 0.84)^2 = 15.68 \approx 16$

(β as the probability of making a Type II error and α as probability of making a Type I error)

z_α = A standardized normal deviate value that correspond to a level of statistical significance equal to 1.96

$z_{\beta=}$ probability of detection or power (80%)

N= Total number of participants

Δ= the Effect Size

Effect sizes as small, moderate, and large (0.2, 0.5, and 0.8 for two-group comparisons)

A guideline by Jacob Cohen – (*Statistical Power Analysis for the Behavioral Sciences, Revised Edition*) (Berkowitz, 1990).

$N=16 / (0.8)^2$

N= 25 per group, Hence a total of 50 subjects were required for the study.

3.4 Study Participants

Study participants were recruited from the Children ward and the Central laboratory of the Korle Bu Teaching Hospital. Parents or Guardians were informed about the background and procedures of the study. Selected participants were recruited into the study after obtaining informed consent from their parents. This study enrolled forty-six (46) participants with malaria infection and thirty-one (31) participants who were not infected were included as controls.

3.4.1 Sampling Method

Random sampling technique was used as the sampling method.

3.4.2 Inclusion Criteria: Malaria positive patients and controls within the age of 6 months- 9 years.

3.4.3 Exclusion Criteria:

- Patients with hypertension, renal diseases, liver disease, and adrenal diseases.
- Those with human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS).

These exclusion criteria's are known to affect the results if not taken into consideration and also ensure the safety of subjects during the study.

3.5 Collection and Processing of Blood Specimen

Five milliliters (5ml) of venous blood was obtained from the anterior cubital vein by sterile venipuncture procedure using 5ml disposable sterile syringe. Four milliliters (4mls) of blood sample were collected into a lithium heparin bottle to assay for lipids and glucocorticoids. The remaining 1ml of blood sample was transferred into di-potassium ethylenediaminetetracetic acid (K_2EDTA) vaccutainer bottles for malaria parasite detection on thick and thin blood films.

3.6 Detection of Malaria Parasite

Thick and thin blood films were made from EDTA blood sample.

3.6.1. Preparation of Phosphate buffer:

0.74g of potassium hydrogen phosphate was weighed into a glass beaker, about 200ml of distilled water was added and stirred. 1.05 g of sodium hydrogen phosphate was weighed

and added to the solution in the beaker and stirred. After the solution was dissolved it was transferred into a volumetric flask and topped up to the 1 L mark. The Phosphate buffer (pH 7.2) was stored in a dark bottle and kept away from sunlight (WHO Malaria standard, 2016).

3.6.2 Stained blood film:

Principle: Thick and thin blood smear are the gold standard method for malaria diagnosis. Giemsa stain is a mixture of methylene blue, azure and eosin dye. Eosin and azure are the acidic dyes that stains the granules and cytoplasm which makes up the basic components of the cells. Methylene blue is the basic dye that stains the nucleus of the cell which is the acidic components of the cell. Giemsa stain is used to differentiate nuclear morphology of platelets, red blood cells, white blood cells and parasites. Giemsa stain is diluted for use with Phosphate buffer to pH 7.0 to 7.2. The pH of the staining solution is important and is adjusted for different fixatives. More acid pH levels give more selective chromatin staining and less cytoplasmic basophilia; less acid pH levels give denser nuclei and increased cytoplasmic basophilia.

3.6.3 Thick blood film:

Three drops of blood were added to clean and dry slide, allowed to dry on a drying rack. The fixing of the thick blood film was done by immersing briefly three (3) times in a Coplin jar containing acetone. The Giemsa stock was diluted 1 in 10 with Phosphate buffer (pH 7.2). The slides were stained with 10% Giemsa stain, washed and air dried for about 30 minutes. A drop of oil was added to the slide and examined under microscope (100x oil immersion). The number of parasites were counted and reported by using the following grading as described by (Ogbodo, 2008).

- 1-999 Parasites/µl – Low Parasitaemia
- 1000- 99,999 Parasites/µl – Moderate
- >100,000 Parasites/µl – High Parasitaemia

3.6.4 Thin blood film:

A drop of blood was placed about 1/3 the length of the slide and by spreader the blood was spread forward with suitable speed to form a thin film. The slide was allowed to air dry for 5 minutes, the slide was fixed with methanol for five minutes and allowed to air dry and stained with 10% Giemsa stain. The slide was washed with water and allowed to dry for 30 minutes, a drop of oil was added to the slide and was examined under microscope (100x oil immersion) (John Hopkins School of medicine, 2004).

Quality control was ensured by covering the microscope when not in use to avoid exposure to dust and avoid fungal growth on the lenses. The microscope slides were free from grease, fungus or moisture and also free of abrasions on the surface.

3.7 Biochemical Analysis of Plasma Lipids

Heparinised blood samples were centrifuged at 5000rpm for 5minutes after which plasma HDL, total cholesterol, LDL and triglycerides were estimated using a chemistry analyzer. A set of tubes were labelled as reagent blank, standard and sample respectively for the procedure of various test. 50µl of distilled water, cholesterol standard and plasma were pipetted into a set of tubes labelled reagent blank, standard and sample respectively and 1000µl of reagent was pipetted into each of the labelled tubes. The labelled tubes were mixed and incubated for 15 minutes at 37^{o}C. 50µl of distilled water, Triglycerides standard and plasma were pipetted into a set of tubes labelled reagent blank, standard and

sample respectively and 1000µl of reagent was pipetted into each of the labelled tubes. The labelled tubes were mixed and incubated for 15 minutes at 37^{o}C.

50µl of distilled water, HDL standard and plasma were pipetted into a set of tubes labelled reagent blank, standard and sample respectively and 1ml of reagent was pipetted into each of the labelled tubes. The labelled tubes were mixed and incubated for 10 minutes at 37^{o}C.

3.7.1 Principle:

A. Total Cholesterol

Total-Chol is measured enzymatically in a series of coupled reactions. The Cholesterol esters are hydrolyzed by cholesterol ester hydrolase into cholesterol, the cholesterol is oxidized into a ketone and hydrogen peroxide by cholesterol oxidase. The generated hydrogen peroxide is decomposed and measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is of the dye measured at 500 nm and is proportional to cholesterol concentration.

The reaction is below:

Cholesterol ester + H_2O Cholesterol ester hydrolase > Cholesterol + fatty acid

Cholesterol + O_2 Cholesterol oxidase > Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-aminophenazone + phenol Peroxidase > Quinoneimine (red) + 4 H_2O

B. Triglycerides

The enzymatic method involves the hydrolysis of triglycerides by lipase into glycerol. Glycerol is then oxidized by glycerol oxidase, hydrogen peroxide in the presence 4-aminophenazone produces a red phenolic derivative by reacting peroxidase. Absorbance

is measured at 500 nm and the intensity of the red color is directly proportional to concentration of triglycerides. The reaction is below:

Triglycerides + $3H_2O$ Lipase > Glycerol + fatty acids

Glycerol + ATP Glycerol kinase > Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 Glycerol phosphate oxidase> dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminophenazone + 4-chlorophenol Peroxidase> Quinoneimine (red) + $2H_2O$ +HCl

C. High density lipoprotein (HDL) cholesterol

Low density and very low density lipoprotein are precipitated by the addition of a reagent containing phosphotungstate and magnesium ions**.** After centrifugation, high density lipoprotein remains in the supernatant fraction and its cholesterol is determined .The reactions are as follows:

HDL-Cholesterol esters Cholesterol esterase hydrolase > HDL-esterified cholesterol + fatty acid

Cholesterol + O_2 Cholesterol oxidase > cholestenone + H_2O_2

H_2O_2 + 4-aminophenazone + phenol Peroxidase > Quinoneimine (red) + H_2O

Absorbance is measured at 500 nm.

D. Low Density Lipoprotein

Low density lipoprotein cholesterol is calculated from measured values of total cholesterol, triglycerides and High density lipoprotein cholesterol.

[Low density lipoprotein-chol] = [total cholesterol] - [HDL-chol] - [Triglycerides/5], [Triglycerides/5] is an estimate of VLDL cholesterol.

3.8 Biochemical Analysis of Plasma Cortisol

3.8.1 Materials

1. A plate reader (405 to 420 nm)
2. Adjustable pipettes
3. Ultra-pure water or double distilled water
4. Cortisol Elisa Kit (Cayman Chemical)

Manufacturer's recommendations were followed in the preparation of materials.

3.8.2 Principle: This assay is based on the competitive binding between cortisol and cortisol -acetyl cholinesterase (AChE) conjugate for a fixed amount of cortisol-specific mouse monoclonal antibody binding sites. The concentration of the cortisol varies while the cortisol tracer is held constant, the amount of cortisol tracer that is able to bind to the cortisol monoclonal antibody will be inversely proportional to the concentration of the cortisol in the well. The free or tracer antibody-cortisol complex binds to the goat polyclonal anti-mouse IgG that has been attached to the well. The plate is washed to remove any unbound reagents, the Ellman's reagent contains the substrate to AChE (which is acetylthiocholine) is added to the well. The products of the enzymatic reaction (thiocholine and 2-nitrobenzoic acid) produces a distinct yellow color and is absorbed strongly at 412nm. The intensity of the color is determined spectrophotometrically and is proportional to the amount of cortisol tracer bound to the well which in inversely proportional to the amount of free cortisol present in the well during incubation.

3.8.3 Pre-Assay Preparation

All the Ultra-pure water used to prepare the ELISA reagents and buffer was deionized and free of trace organic contaminants. The pre-assay for all the reagents are below.

Cortisol AChE tracer and Tracer dye

1 vial of the Cortisol AChE was reconstituted with 6ml ELISA buffer, 60 μl of the tracer dye was added to 6ml tracer and mixed thoroughly.

Cortisol ELISA Monoclonal Antibody

1 vial of the Cortisol ELISA monoclonal antibody was reconstituted with 6ml ELISA buffer, 60 μl of the antiserum dye was added to 6ml tracer and mixed thoroughly.

Buffer Preparation

1. ELISA Buffer Preparation

The ELISA buffer concentrate was diluted with 90ml of Ultra-pure water.

2. Wash Buffer Preparation

5ml of the wash buffer concentrate was diluted to a total volume of 2 liters with Ultra-pure water and 1ml of polysorbate 20 was added.

3.8.4 Preparation of Cortisol Elisa Standard

Serial dilution of the stock standard solution was made as follows:

100μl of the cortisol ELISA standard was transferred into a clean test tube and then diluted with 900 μl of Ultra-pure water. Eight clean test tubes were numbered from one to eight (1-8) .900 μl of the ELISA buffer was transferred into the test tube #1 and 600 μl

of the ELISA buffer was transferred to tubes #2-8. 100μl of the stock standard was transferred to test tube #1 and mixed thoroughly. Serial dilution of the standard was done by removing 400μl from tube #1 and transferring to tube #2 and was mixed thoroughly. 400μl was transferred from tube #2 to tube #3 and was mixed thoroughly. This process was repeated for tube #4 to tube #8. The diluted standards were not stored for more than 24 hours.

3.8.5 Performing the Assay

Addition of Reagents to the wells

100μl of the ELISA buffer was added to the non-specific binding well (NSB) and 50μl of the ELISA buffer was added to the B_o well.

50 μl from tube #8 of the cortisol ELISA standard was added to both of the lowest standard wells (S8),another 50 μl from tube #7 of the cortisol ELISA standard was added to next two standard wells (S7) . This process was repeated for tube #6 to tube #1.

50 μl of the plasma was added to the sample per well and 50 μl of the Cortisol AChE was added to each well except Total activity (TA) and blank wells.

50 μl of Cortisol ELISA monoclonal antibody was added to each well except Total activity (TA), Non-Specific Binding well (NSB) and blank well.

Incubation of Plate

Each plate was covered with a plastic film and incubated overnight at 4^0C.

1	2	3	4	5	6	7	8	9	10	11	12
Blank	S1	S1									
Blank	S2	S2									
NSB	S3	S3									
NSB	S4	S4									
B_O	S5	S5									
B_O	S6	S6									
B_O	S7	S7									
TA	S8	S8									

Figure 5: Sample Plate Format

TA – Total Activity

NSB- Non-Specific Binding

B_o- Maximum binding

4-12 –Samples

3.8.6 Development of Plate

20 ml of Ellman's reagent was reconstituted with 20 ml of Ultra-pure water; .the wells were emptied and rinsed five times with wash buffer. 200 μl of Ellman's reagent was added to each well and 5 μl of the tracer was added to the Total activity wells (TA).

The plate was covered with a plastic film and allowed the plate to develop in the dark. Optimum development is obtained in the dark; exposure to light would affect the results.

Reading the Plate

The bottom of the plate was wiped with clean tissue to remove dirt and fingerprints.

The plate cover was carefully removed to prevent any loss of the Ellman's reagent which will affect the absorbance readings. The plate was read at a wavelength of 420 nm, the absorbance was checked periodically until the B_o well reached a minimum of 0.3 absorbance units after the blank subtraction.

3.8.7 Calculation of Sample Concentration

The average absorbance of Maximum Binding (B_o) and Non-Specific Binding (NSB) was calculated. The correct maximum binding (Corrected B_o) was calculated by subtracting the average absorbance of Non-Specific Binding (NSB) from the Maximum Binding (B_o). The B/B_o (Sample Bound/Maximum Bound) was calculated by subtracting the average NSB absorbance from the S1 absorbance (standard well) and then divided by the corrected B_o.The standard wells S2- S8 and all sample wells were calculated by subtracting the average NSB from the respective wells and dividing through by the corrected B_o.The values for B/B_o (Sample Bound/Maximum Bound) were multiplied by 100 to obtain $\%B/B_o$.

3.8.8 Plotting of the Standard Curve and Sample Concentration

The $\%B/B_o$ (Sample Bound/Maximum Bound) for standard S1-S8 was plot against the Cortisol concentration using a linear (y) and log(x) axes, a 4-parameter logistic was performed. The concentration of each sample was obtained by using the equation obtained from the standard curve plot.

Quality control was ensured by covering the microplate reader when not in use to avoid exposure to dust and avoid fungal growth on the lenses. The wells were free from grease, fungus or moisture and also free of abrasions on the surface. The plate was covered with

a plastic film and allowed the plate to develop in the dark, Optimum development is obtained in the dark; exposure to light would affect the result.

3.9 Statistical Analysis

Data was entered into a computer and analyzed using the IBM SPSS20 (Statistical Package for Social Sciences).

- . The descriptive statistics (mean, median and standard deviation) for continuous variables and proportion for categorical variables were determined.
- Chi-Square was used to compare the Sociodemographic characteristics between the participants.
- Association between cortisol and parasite count were by Spearman correlation.
- The level of statistical significance was set as $p \leq 0.05$.

3.9.1 Normality, Skewness and Kurtosis of Lipids

A Shapiro-Wilk's test ($p>0.05$) (Shapiro & Wilk, 1965) and a visual inspection of their histogram, normal Q-Q plots and box plots for the participants show that LDL-Chol levels, HDL-Chol, Total-Chol, VLDL-Chol and Triglycerides were not normally distributed for both controls and cases. LDL-Chol showed a skewness of 0.77(S.E=0.27) and a kurtosis of 1.46(S.E=0.54). Triglycerides showed a skewness of 0.63(S.E=0.27) and a kurtosis of 1.40 (S.E=0.54). HDL-Chol showed a skewness of 0.80(S.E=0.27) and a kurtosis of 0.88(S.E =0.54). VLDL-Chol showed a skewness of 1.85(S.E=0.27) and a kurtosis of 4.08 (S.E=0.54). Total-Chol showed a skewness of 0.22 (S.E=0.27) and a kurtosis of 0.94 (S.E=0.54).

CHAPTER FOUR

RESULTS

4.1. Sample Characteristics of Study Population

A total of 77 participants were investigated in the study. Out of the 77 participants 46 were positive (59.7%) for malaria and 31 were negative (41.35%) for malaria. The numbers of males were 40 (51.9%) and females were 37 (48.1%). The mean age and standard deviation was 6 ± 2 years (Table 4.2).

4.1.1 Prevalence of Malaria

The prevalence of malaria in this study was 59.7% (46 participants). The prevalence due to gender was 54.3% (25 participants) for males and 45.7% (21 participants) for females. The age range 1-5 years had a prevalence of 23.9% (11 participants) and 6-9 years prevalence was 76.1% (35 participants).

4.2. Sociodemographic Characteristics of Participants

Seventy-seven (77) participants aged 1 to 9 years participated in the study, this was made up of a male group with the highest number of participants of 40 (51.9 %) and female participants were 37 (48.1%). Participants who did not attend school were 12 (15.6%) with 65 (84.4%) of participants having primary school education (Table 4.1).

Table 4.1: Socio-demographic characteristics of participants

Variable	Median ± S.D	Frequency	Percent (%)
Age	7±2	77	100
Gender			
Male		40	51.9
Female		37	48.1
Educational Level			
No School		12	5.6
Primary		65	84.4
Residence of Participants			
Korle Bu		15	19.5
Outside Korle Bu		62	80.5

Table 4.1 shows the socio-demographic characteristics of the study population. Values are expressed as mean, median, frequencies and standard deviation.

4.3 Sociodemographic Characteristics of Participants associated with Malaria

The results in (Table 4.2), shows that age groups were significantly associated with malaria. Children between within 6-9 years were more likely to get malaria than those within 1-5 years group. Gender and educational level were not significantly associated with malaria (Table 4.2).

Table 4.2:Socio-demographic characteristics of participants associated with Malaria

Variable	Positive	Negative	OR (95% CI)	P-value
Gender				
Male	25(54.3%)	15 (48.4%)	1.123(0.716-1.761)	**0.608**
Female	21 (45.7%)	16 (51.6%)	1	
Educational Level				
Primary	41(89.1%)	24(77.4%)	1	
No School	5(10.9%)	7(22.6%)	0.481(0.168-1.380)	**0.165**
Age (Years)				
1-5	11(23.9%)	19 (61.3%)	1	
6-9	35(76.1%)	12(38.7%)	1.966(1.226-3.150)	**0.001***
Bed net				
Yes	19(41.3%)	4(12.9%)		**0.008***
No	27(58.7%)	27(87.1%)		

Table 4.2 shows the socio-demographic characteristics of participants and their association with parasitaemia. $p<0.05$ indicates significance. Odds Ratio (OR) and Confidence interval (C.I). Positive: Presence of malaria parasite in blood, Negative: Absence of malaria parasite in blood

4.4 Biochemical Measurement

Table 4.3 shows the mean and median values of the lipid profile and cortisol in study participants.

Table 4.3: Biochemical Measurement of Lipids and Cortisol in study participants

Variable	Median ± S.D	Frequency	95% CI of Median	
			Upper	Lower
LDL-Chol mmol/L	2.38±0.58	77	2.63	2.19
HDL-Chol mmol/L	0.76±0.51	77	1.11	0.52
Total-Chol mmol/L	3.62±0.88	77	3.78	3.37
VLDL-Chol mmol/L	0.34±0.18	77	0.38	0.31
Triglyceride mmol/L	1.76±0.81	77	2.03	1.11
Cortisol (pg//ml)	0.54±0.042	77	0.55	0.53

Table 4.3 shows levels of lipid profile and cortisol among study participants. Values are expressed as Median ± SD and confidence interval (C.I) of the variables.

4.5. Association of Malaria Count with Lipid Profile values in cases

HDL-Chol was negatively correlated with the malaria count and was significant (rho=-0.5380, $p<0.0001$). Triglyceride was positively but weakly correlated with malaria count (rho=0.2967, $p<0.05$). Total-Chol, VLDL and LDL cholesterols did not associate with level of parasitaemia ($p>0.05$) (Table 4.5).

Table 4.4: The relationship between malaria count and lipid profile in cases

	Variable 1	Variable 2	p-value	rho
Lipids	**Median±S.E (**mmol/L)	**Malaria Count** (Parasites/µl)		
HDL-Chol	0.44±0.22	15998	<0.0001	- 0.5380
Total-Chol	3.005±0.59	15998	0.7226	-0.05378
Triglycerides	2.18±0.54	15998	<0.05	0.2967
LDL-Chol	2.15±0.55	15998	0.3331	0.1460
VLDL-Chol	0.33±0.18	15998	0.7502	0.04824

Table 4.4 shows associations between several correlates and parasitaemia. Rho is Spearman Correlation Coefficient. $p<0.05$ indicates significance

4.5.1. Comparison of Malaria Count and Cortisol Cases

Table 4.5 shows the association between cortisol and malaria count. There were no association between the two groups ($p>0.05$).

Table 4.5: Malaria Count and Cortisol Cases

Variable 1	Variable 2	p-value	rho
Cortisol (pg//ml)	**Malaria Count** (Parasites/µl)		
0.55±0.03	15998±4067	0.5379	-0.093

Table 4.5 shows association of cortisol with malaria count. Rho is Spearman Correlation Coefficient. $p<0.05$ indicates significance

4.5.2 Comparison of Malaria Positive and Negative of Cortisol and Lipids

All the biochemical parameters including Cortisol, LDL-C, HDL-C, Triglycerides and Total-C cases and controls were not significantly different (p >0.05) among malaria positive and negative subjects and is shown (Table 4.7).

Table 4.6: Biochemical Parameters of Malaria Positive and Negative

Biochemical Parameter	**Positive (N=46)** (mmol/L) **Median ±SD**	**Negative (N=31)**	**p-value**	**rho**
Cortisol pg/ml	0.55±0.03	0.50±0.04	0.5379	0.0045
LDL-C mmol/L	2.15±0.55	2.78±0.46	0.9438	0.013
HDL-C mmol/L	0.44±0.22	1.28±0.32	0.9518	0.011
Triglycerides mmol/L	2.18±0.54	0.75±0.46	0.1217	0.28
VLDL-C mmol/L	0.33±0.18	0.37±0.19	0.5951	0.099
Total-C mmol/L	3.005±0.59	4.51±0.56	0.6985	-0.072

Table 4.6 shows that values are expressed as median, standard deviation (Median ± S.D) and Correlation Coefficient (rho). P<0.05 indicates significance level between groups comparison. P>0.05 indicates no significant difference between groups comparison.

4.5.3 Association of Cortisol with categorized parasitaemia

Parasite counts of cortisol cases were further grouped into three; High parasitaemia (>100,000 Parasites/µl), Moderate (1000-99,999 Parasites/µl) and low parasitaemia (1-999 Parasites/µl). A total of 38 (82.61%) participants had high parasitaemia (1000-99,999 Parasites/µl), 6 (13.04%) participants had low parasitaemia (1-999 Parasites/µl) and 2 (4.35%) participants had high (mortality) parasitaemia. The median of the cortisol among High, Low and Moderate parasitaemia did not vary significantly (p >0.05) (Table 4.7).

Table 4.7: Cortisol cases and Parasitaemia Levels

Parasitaemia Level	**Median ±SD Cortisol (mmol/L)**	**C.I (Median) Cortisol**	**Minimum**	**Maximum**	**N**
High (>100,000 Parasites/µl)	0.56±0.007	0.55 to 0.56	0.55	0.56	2
Moderate (1000-99,999 Parasites/µl)	0.55±0.03	0.54 to 0.56	0.39	0.57	38
Low (1-999 Parasites/µl)	0.56±0.01	0.54 to 0.57	0.54	0.57	6
				P value>0.05 (0.3599)	

Table 4.7 shows the association of high, low parasitaemia and high mortality with cortisol levels. Values are expressed as median and standard deviation (Median ± S.D), Number of samples (N). p<0.05 indicates statistical significance.

4.5.4 Association of cortisol and malaria count

The graph in Figure 6 and 7 shows the relationship between cortisol and level of parasitaemia ($p > 0.05$).

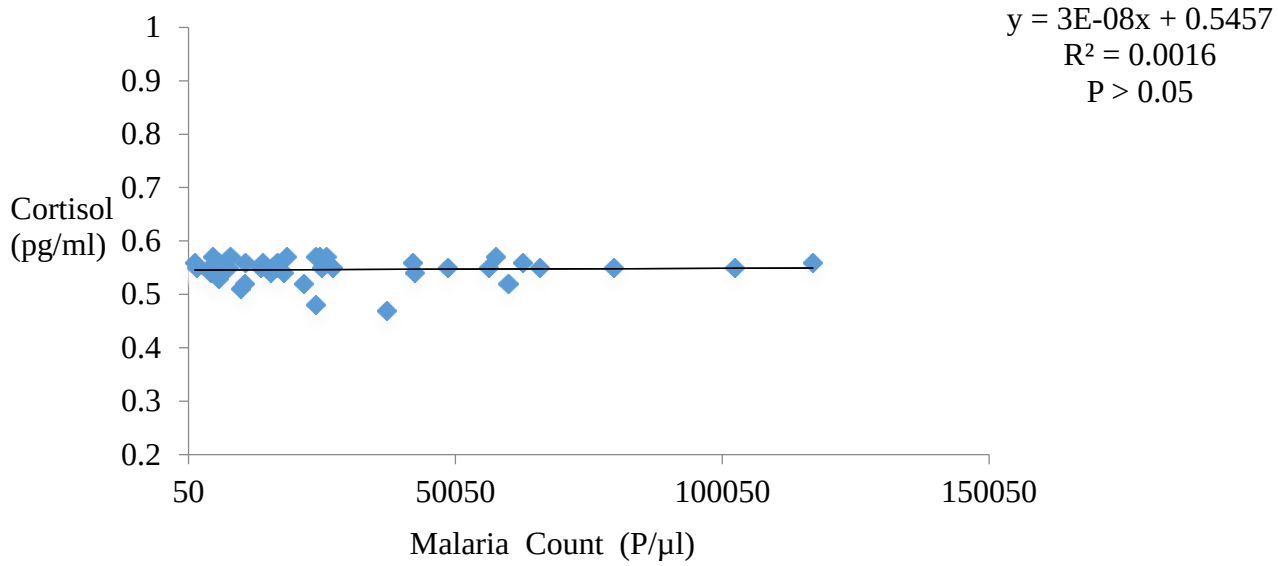


Figure 6: Association of Cortisol with Malaria count (High Parasitaemia)

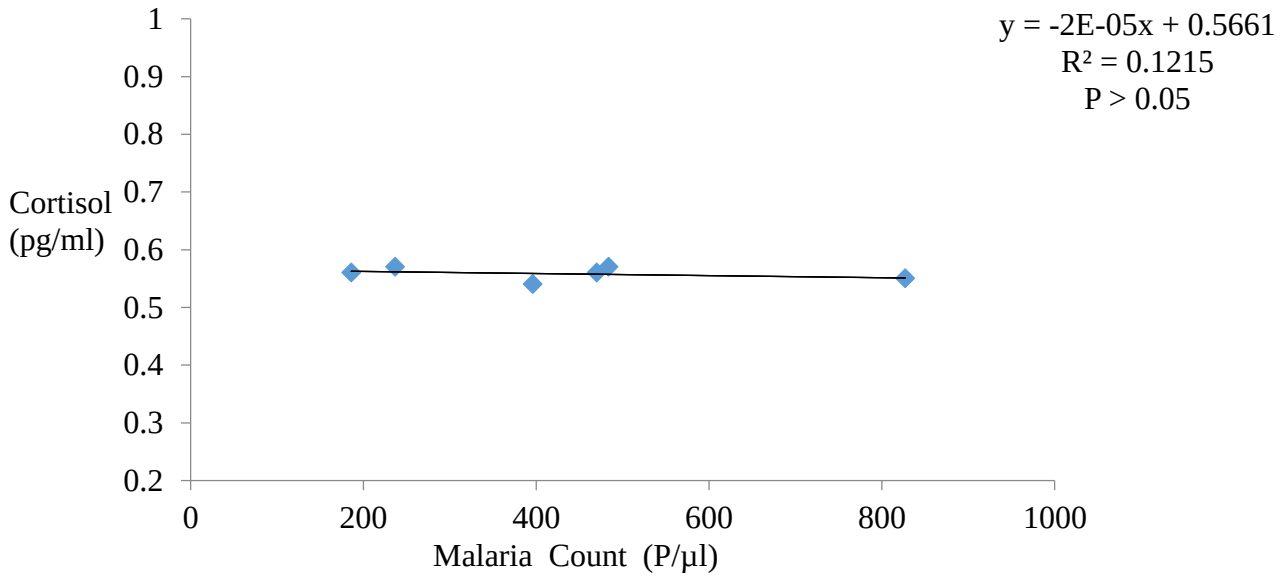


Figure 7: Cortisol and Malaria count (Low Parasitaemia)

4.5.5 Comparison of mild and severe malaria of Lipid cases

There was no significant difference between mild malaria and severe malaria with lipids in case group ($p>0.05$) (Table 4.8).

Table 4.8: Comparison of mild and severe malaria with Lipids

Variable (mmol/L)	Mild (n=6)		Severe (n=40)		
	Median ±SD	(C.I) Mild	Median ±SD	(C.I) Severe	p-value
TG	1.84±0.56	1.24 to 2.66	2.24±0.54	2.03 to 2.34	0.91
HDL	0.87±0.11	0.65 to 0.93	0.42±0.18	0.35 to 0.47	0.57
LDL	2.19±0.36	1.78 to 2.76	2.13±0.58	1.96 to 2.45	0.41
VLDL	0.41±0.13	0.28 to 0.63	0.32±0.19	0.29 to 0.39	0.98
Total-C	3.64±0.38	2.82 to 3.81	2.94±0.59	2.77 to 3.36	0.29

Table 4.8 shows the comparison of mild and severe malaria with lipids. $p<0.05$ indicates significance level.

CHAPTER FIVE

DISCUSSIONS

Malaria remains a major global and national health problem. Despite the malaria eradication programmes, treatment and management of the disease, there are challenges faced by the health sector and government. This study is aimed at evaluating the impact malaria has on lipids and glucocorticoids.

The prevalence of malaria in this study was 59.7%. In this study the prevalence of malaria due to gender was higher for males than for females (Table 4.1). A study by Onwe et al., (2018) showed that males had a high prevalence of malaria infection than females in a study in Nigeria.

Participants within 6-9 years age range had a high prevalence (Table 4.2) and were more susceptible to malaria infection than the 1-5 years age group. This study agrees with a study by Onwe et al., (2018) stating that children within the age range of 0-5 years had low prevalence because they are under close watch by their parents and majority of them sleep under treated mosquito nets, whiles 6-9 years have a high prevalence because some stay out late in the night and also expose their body which makes them vulnerable to malaria infection. Children between the ages of 1-5 years had the lowest prevalence due to the higher attention given to them by their parents as compared to children between 6-9 years. Children within the age range of 6-9 years are more adventurous and aggressive than children within the age of 1-5 years which makes them vulnerable to malaria infection.

The national malaria control programme (NMCP) used mass media campaigns to advocate, create awareness and intensify education on treatment and test of malaria, a total number of 54TV stations and over 1805 radio adverts were aired across Ghana (G.H.S., 2017). This study shows that a lot of participants did not have bed net as compared to those who have a bed net (Table 4.2), despite the publicity and awareness from national malaria control programme malaria is still a major health problem.

There was no relationship between levels of Cortisol and malaria count in the case group. It has been suggested that malaria infection decreases the synthesis of cortisol receptors which increases plasma glucocorticoids, thus leads to the negative feedback from cortisol which leads to the activation of the HPA axis, thus stimulate the release of ACTH for the production of cortisol. A study by Tayeb, (2015) revealed that patients with high parasitaemia had high levels of cortisol and there was a significant difference compared to the control group. In this study there was no significant difference in cortisol levels in malaria patients in comparison with the control group, although the median of cortisol cases was higher than the control group. However, it was reported by Ibrahim et al., (2011) there was no significant difference in mean values of total cortisol levels in malaria patients in comparison with the control group in eastern Sudan. In this study, Malaria parasite counts were further grouped into three categories (Table 4.7): High, Moderate and Low parasitaemia and compared with their cortisol levels. There was no significant difference between the groups and no association with cortisol.

There was a significant difference in the median values between HDL-Chol and malaria count of the cases. Malaria parasites modify the metabolic pathway of lipids in the liver and also the oxidation of lipoproteins for the formation of their membrane which

accounts for the degradation of lipoprotein (Ogbodo, 2008). This may explain the negative correlation with HDL-Chol and Malaria count (Table 4.4): HDL-Chol was decreased whiles the malaria count was increased.

In this study plasma levels of LDL-Chol, VLDL-Chol, Total-Chol and HDL-Chol were lower than the control subjects (Table 4.6). According to Ogbodo, (2008) oxidation of lipoproteins such as LDL and HDL increases the cytoadherence of infected red blood cells which consequently increases the pathogenicity of the disease in children. Malaria parasites take advantage of the oxidation of the lipoprotein which leads to increased replication of parasite and the severity of the disease. The decrease in Total-Chol may be as a result of a decrease in HDL-Chol due to oxidation of lipoproteins and also the oxidation of HDL-Chol leads to the decrease in activity of lecithin cholesterol acetyl transferase which leads to impairment of HDL metabolism (Ogbodo, 2008). Impairment of HDL metabolism results in less removal of LDL in circulation, comparing the ratio of HDL and LDL levels in malaria infected patients with impaired HDL metabolism may result in coronary heart disease.

LDL-Chol, VLDL-Chol and Total-Chol and malaria count of the cases were not significantly associated. This is because of the oxidation HDL-Chol and acute phase response, which leads to the decrease in activity of the lecithin cholesterol acetyl transferase, consequently produces LDL-Chol and impairment of HDL. A study by Dias et al., (2016) showed that Total-Chol, HDL-Chol and LDL-Chol in *plasmodium falciparum* malaria patients were lower than the controls and also stated that the changes in plasma lipids could be as a result of acute phase reaction in malaria infection.

There was no significant difference between the lipid profile case and control groups, although they showed relative decrease when compared to the control group. However it is been reported by Jacob, (2014) that plasma levels of Triglycerides, HDL-Chol LDL-Chol and Total-Chol were significantly low in children infected with *plasmodium falciparum*.

In this study plasma levels of triglycerides were higher than the control groups (Table 4.6). There was a significant association between triglycerides and malaria count of the case group (Table 4.4). Acute phase response leads to the decrease in activity of the lecithin cholesterol acetyl transferase and also the impairment of HDL-Chol metabolism which further leads to increase in triglycerides. A study by Jacob, (2014) showed that acute phase reactions or response is associated with changes in lipids which includes a decrease in HDL-Chol,LDL-Chol and an increase in Triglycerides. The reason for the increase in triglycerides is the increase in oxidation of HDL-Chol and acute phase response. Studies by Warjri et al., (2016), Gazin, (2004) showed that triglycerides were higher than the control groups and suggested that hypertriglyceridaemia can be used as indicator of severe malaria. A study by Dias et al., (2016) revealed that malaria parasites use the cholesterol and phospholipids from the host for their normal growth and development, lipids plays a vital role in the life cycle of the parasites by providing metabolic necessities and it explains the decrease in HDL-Chol ,Total-Chol and LDL-Chol. In contrast to our study, an earlier study by Gazin, (2004) revealed that triglycerides were significantly higher in severe malaria than mild malaria.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study showed no association between cortisol and malaria in Ghanaian children under 10 years. The study showed a significantly inverse association between HDL-Chol, with malaria. Triglycerides associated positively with parasitaemia. Children 6-9 years were more susceptible to malaria infection than 1-5 year old. The impact of malaria on glucocorticoids merit further studies.

6.2 Recommendations

Recommendations for the research findings

1. More effort is needed in reducing the prevalence of malaria in the country by distribution of Insecticide treated nets, effective treatment and diagnosis of malaria in children under ten (10) years.
2. An intense research on the Hypothalamus-Pituitary-Adrenal axis (HPA axis) of children under ten years with malaria infection.
3. An intense research on Lipid modifications (oxidized, glycated and small dense) and measurement of immune markers (cytokines).

6.3 Study Limitations

The sample size was a limitation in the study possibly due to the period of sample collection as the prevalence of malaria increases during the rainy season (May-July) in Ghana.

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APPENDICES I

PARTICIPANTS INFORMATION SHEET

Title: The Impact of Malaria on Lipids and Glucocorticoids.

Principal Investigator: Prof. Henry Anane Asare (Head of Department –Chemical Pathology).

Information :(To be read or translated to patients that meet study criteria in their own mother tongue by study personnel (Principal Investigator or research assistant).

Dear Volunteer,

This consent form contains information about the research titled **"The Impact of Malaria on Lipids and Glucocorticoids".**

In order to be sure that you are duly informed about your participation in this research, I am asking you to read (or have read to you) this Consent Form. You will also be asked to sign it (or thumbprint in front of a witness). I will give you a copy of this form. This Consent form might contain some words that are unfamiliar to you. Please ask me to explain anything you may not understand.

Why is this study planned?

You are being asked to participate in the above study in order to look at the baseline level of some laboratory tests used to detect malaria as well as to determine its impact on lipids and glucocorticoids. This study also seeks to establish the link between malaria infection, lipids and glucocorticoids. I would follow up on you to record all events that occur during the malaria infection.

Procedure to be followed:

During the procedure, blood will be collected via venipuncture from your child by trained Scientist/Technician. A total of 5ml (two table spoons) of blood will be taken. The blood sample will be used for analysis to determine the presence of malaria and its impact on lipids and corticosteroids.

Discomfort/Risk:

The risks in this study are minimal (i.e. Slight pain during the insertion of the needle into the vein and could make the child cry). There are no foreseeable dangers to either you or your child in this study during the processing of your child's blood sample.

Benefit:

There is no direct benefit to you or your child. However, this study will help to enhance knowledge on how to care for children that are infected with malaria.

Statement of Confidentiality:

All data/records will be kept strictly confidential and will only be made available to the research team. If the results of this study are published, the data generated will be presented in group form and individual children will not be identified. Information about your child that will be collected from the study will be stored in a file which will not have your child's name on it, but a number assigned to it.The number assigned will not be disclosed to anyone except the principal investigator or the research team.

Voluntary Participation:

Your child's participation is voluntary. If you feel your child has in any way been forced into participation, please inform the lead researcher. If at any point in time you choose to

withdraw from the study, you are at liberty to and it does not affect the services been provided to your child.

Contact information:

Prof. Henry Anane Asare (Head of Department –Chemical Pathology)

(Principal Investigator) Contact: 0246024002

Benedict Nii Ayi Armah

(Lead Researcher) Contact: 0572559185

CONSENT FORM

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	YES / NO
I have been given sufficient time to consider whether or not to participate	YES / NO
in this study.	
I am satisfied with the answers I have been given regarding	YES / NO
the study.	
I understand that taking part in this study is voluntary (my choice)	YES / NO
and that I may withdraw from the study at any time without this	
affecting my medical care.	
I consent to the research assistant collecting and processing my child's Information, including information about his/her health.	YES /NO

VOLUNTEER AGREEMENT:

Declaration by Parent/Guardian:

I……hereby consent to take part in this study. I understand the nature, risk and benefits of the study and that I may withdraw at any time.

…….. Date …….

Signature of Participant/Thumbprint

……. Date …….

Signature of Witness/Thumbprint

…… Date …….

Signature of Investigator/Thumbprint

APPENDICE II STUDY QUESTIONNAIRE –DATA SHEET

TITLE OF STUDY: IMPACT OF MALARIA ON LIPIDS AND GLUCOCORTICOIDS

1. Name ……..

2. Serial Number …… 3. Age ……

4. Occupation …… 5. Gender ……

6. Educational Background [Tick the appropriate one]

a. JHS ( ) b.SHS ( ) c.Tertiary ( ) d.Vocational ( ) e. Primary ( ) f. None ( )

7. Marital status …… 8. Number of Children ……..

8. Do you own a bed net? YES [ ] NO [ ]

9. What is the current condition of your treated net: In good order [ ] Torn [ ]

10. Reasons for not using the mosquito treated net

a) Housing structure

b) No bed

c) Bed nets do not prevent malaria

d) It is toxic

e) Weather

f) Other –

13. Can you tick signs and symptoms of malaria?

a) Fever

b) Headache

c) Diarrhoea

d) General body weakness

e) Vomiting

14. What do you do when children under ten years contract malaria, where are they treated?

a) Hospital

b) Traditional herbalist

c) Self medication

d) Others-

15. Housing environment

a) Proximal to breeding sites :(old tires, containers, ponds ,Factories, stagnant water)

b) Clean environment

c) Farming activities

d) Others

ETHICAL CONSIDERATION

Approval was taken from the College of Health Sciences-University of Ghana (Legon). Consent forms were taken from all parents or their guardians before being enrolled in the study. All parents or guardians were informed on the nature of the study before the questionnaire was administered. Confidentiality was observed and unauthorized persons will not have access to the data collected.

Each participant was assigned a study identification number, participants name will not be released outside the research group. Codes were used and no identification was made for the participant. Participants were informed that their data will used anonymously and that the aim of the study is to understand the impact of malaria on lipids and corticosteroids.

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/FEB/2019 February 08, 2019

Benedict Nii Ayi Armah
Department of Chemical Pathology
School of Biomedical and Allied Health Sciences
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.6 – 5.13/2018-2019***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its February 08, 2019 full board meeting reviewed and approved your re-submitted research protocol.

Title of Protocol: **"The impact of Malaria on Lipids and Corticosteroids"**

Principal Investigator: **Mr. Benedict Nii Ayi Armah**

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till February 10, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....

Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Department of Chemical Pathology

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