EVALUATION OF RENAL AND HEPATIC DYSFUNCTION AMONG CHILDREN LESS THAN FIVE YEARS WITH MALARIA IN JASIKAN DISTRICT, OTI REGION- GHANA

BY

ISAAC DJAN ASARE

(10638390)

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JULY, 2019
I hereby declare that except for references to other people’s work, which has accordingly been acknowledged, the afore mentioned thesis is the result of my own research performed at the Department of Chemical Pathology, School of Biomedical and Allied Health Sciences, University of Ghana, Accra, Ghana, and Jasikan District Hospital, Jasikan-Oti region, under the supervision of Professor, Henry Asare-Anane (Head of Department, Chemical Pathology) and Dr. Emmanuel K. Ofori (Department of Chemical Pathology). Neither all nor parts of this thesis have been submitted for another degree elsewhere.

ISAAC DJAN ASARE  ...................................  .................
(MPHIL. CANDIDATE)          SIGNATURE       DATE

PROF. HENRY ASARE-ANANE  ...................................  .................
(SUPERVISOR)          SIGNATURE       DATE

DR. EMMANUEL K. OFORI  ...................................  .................
(SUPERVISOR)          SIGNATURE       DATE
DEDICATION

I dedicate this thesis to the Lord Almighty for His unconditional love, kindness, protection and wisdom throughout this thesis. I also dedicate this work to my wife Alice Djan-Asare and my children (Ann-Mikayla and Areela-Ann) for their immense support, prayers and encouragement.

I further dedicate this work to my father, mother and siblings for their ceaseless prayers and support in the attainment of academic and spiritual heights.
To God be the glory great things He has done and greater things will He do. The Lord directed my paths throughout this thesis and all I am and will be is His. May His name be praised.

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<tr>
<td>ACT</td>
<td>Artemisinin-based Combination Therapy</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate transaminase</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>CSA</td>
<td>Chondroitin Sulfate A</td>
</tr>
<tr>
<td>DGKC</td>
<td>German Society of Clinical Chemistry</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated Intravascular Coagulopathy</td>
</tr>
<tr>
<td>GDHS</td>
<td>Ghana Demographic and Health Survey</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-Macrophage Colony-Stimulating Factor</td>
</tr>
<tr>
<td>GPT</td>
<td>Glutamate Pyruvate Transaminase</td>
</tr>
<tr>
<td>HRP</td>
<td>Histidine Rich Protein</td>
</tr>
<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemist</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>IPT</td>
<td>Intermittent Preventive treatment</td>
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<td>ITN</td>
<td>Insecticide Treated net</td>
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<td>KIM-1</td>
<td>Kidney Injury Molecule-1</td>
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<td>LDH</td>
<td>Lactate Dehydrogenase</td>
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<tr>
<td>MANOVA</td>
<td>Multiple Analysis of Variance</td>
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<td>MARF</td>
<td>Malaria Acute renal failure</td>
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<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>MDH</td>
<td>Malate Dehydrogenase</td>
</tr>
<tr>
<td>MICS</td>
<td>Multiple Indicator Cluster Survey</td>
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<tr>
<td>MSP-1</td>
<td>Merozoite Surface Protein</td>
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<tr>
<td>NAD</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide Adenine Dinucleotide (reduced)</td>
</tr>
<tr>
<td>NGAL</td>
<td>Neutrophil Gelatinase-Associated Lipocalin</td>
</tr>
<tr>
<td>NGO</td>
<td>Non-Governmental Organization</td>
</tr>
<tr>
<td>NMCP</td>
<td>National Malaria Control Programme</td>
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<tr>
<td>OPD</td>
<td>Out-Patient Department</td>
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<tr>
<td>P-5-P</td>
<td>Pyridoxal-5-Phosphate</td>
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<tr>
<td>PfEMP 1</td>
<td>Plasmodium falciparum Erythrocyte Membrane Protein-1</td>
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<tr>
<td>pH</td>
<td>Power of Hydrogen</td>
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<tr>
<td>PV</td>
<td>Parasitophorous Vacuole</td>
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<tr>
<td>RBC</td>
<td>Red blood cell</td>
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<tr>
<td>SCE</td>
<td>Scandinavian Society of Clinical Chemistry</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<td>STATA</td>
<td>Statistics and Data</td>
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<tr>
<td>TNF-α</td>
<td>Tumor Necrosis factor alpha</td>
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<tr>
<td>UNICEF</td>
<td>United Nations International Children’s Emergency Fund</td>
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Background

Malaria continues to be a menace in many parts of the world, contributing to high morbidity and mortality especially in developing countries. Irrespective of the immense contributions made to control malaria infection rates, the infection perpetually plagues numerous lives in sub-Saharan Africa. Malaria infection has also been implicated in renal and hepatic dysfunction. The renal dysfunction typifies an increase of urea and creatinine levels in serum whiles the hepatic dysfunction signifies an increase in liver transaminases. Despite the linkage of malaria with kidney and liver dysfunction, their precise interrelationship or interaction especially in children has not been fully elucidated.

General aim

This study aimed to evaluate the effects of malaria infection on renal and hepatic function among children less than five years in Jasikan, Oti- region, Ghana.

Methodology

A cross-sectional study involving 400 children aged 6-59 months. Participants were tested for malaria parasites using microscopy examination on blood films. Subjects that were positive for the parasite were further categorized into two groups based on the quantification of parasites per micro liter (µl) of blood: children with mild infection (parasitaemia less than 100,000 parasites per microlitre of blood) and those with severe infection (parasitaemia more than 100,000 parasites per microlitre of blood). Malaria negative samples were screened and served as controls. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and bilirubin concentrations were measured to evaluate liver dysfunction whilst serum urea and creatinine were used to assess kidney dysfunction among all participants.
Results

Results show significant difference ($p<0.05$) in serum creatinine, urea, AST and ALT levels of children with positive parasitaemia compared with the control group. Among the positive parasitaemia subjects, urea, creatinine, AST and ALT levels were also statistically different ($p<0.05$) for severe versus mild groups. Serum protein and albumin levels were significantly higher ($p<0.05$) in the control group relative to the case group. The serum total and direct bilirubin levels were significantly higher ($p<0.05$) in the severe infected group relative to the mild and control groups.

Conclusion

This study observed that malaria infection has a significant effect on renal and hepatic functions with increasing concentrations paralleling severity of parasitaemia.
CHAPTER ONE

1.0 Introduction

Malaria is a disease that originated from Italian, which connotes ‘bad air’ called mesh fever on account of its association with swampy areas in humid regions across the globe (Dutta, 2015). Malaria infection is a global and public health issue with an estimated 219 million cases resulting in 435,000 deaths in the year 2017, of which 90% of the cases and 93% of the mortalities were predominant in sub-Saharan Africa (WHO, 2018). According to WHO (2018), the disease accounted for 61% of mortalities among children less than five years in the sub Saharan region. The significant inference that accounts for the malaria infection in the sub-Saharan Africa is principally the climatic alterations that support the proliferation of the vector and growth of the parasite within the vector as well as the host (Ross and Smith, 2010).

In Ghana, malaria is regarded as an endemic disease and a public health problem, as a result of the significant death toll associated with it (Yao et al., 2018). Malaria infection is more prevalent in children and pregnant women and contributes to the high morbidity and mortality in highly endemic areas (Zaki et al., 2013; Gomes et al., 2011). The Global malaria morbidity and mortality were reduced by 37% and 60% respectively in the period between 2003 and 2015 due to a concerted effort by international organizations, Non-Government Organizations (NGO) and governments (Afoakwa et al., 2018). Irrespective of the high investment to control the disease, malaria infection among children in Ghana relative to the global phenomenon has not reduced (Afoakwa et al., 2018). Regrettably, malaria cases in the country continued to rise especially among children less than five years and accounts for over 50% of Out Patient Department (OPD) cases from 2010 to 2012 (Afoakwa et al., 2018). The ailment accounts for 25 per cent of under-five mortality.
Each episode of malaria infection often lasts 5-15 days, coupled with the incapacitation of the individual (Kepha et al., 2016).

The malaria parasite that predominantly infects man in sub-Saharan Africa is the *Plasmodium falciparum* and accounts for 99% of estimated malaria cases in 2016 (WHO, 2017), and accounts for the high mortality and morbidity cases in the sub-region. *Plasmodium falciparum* parasite alternate from less symptomatic in nature to multi-organ phenomenon (Zaki et al., 2013). Malaria infection however has been implicated as one of the factors causing renal and hepatic dysfunction in sub-Saharan Africa (Mishra et al., 2003; Ogbadoyi & Gabi, 2007). It is assumed as complicated malaria when one of the following clinical features is noted: repeated generalized convulsion, jaundice, renal failure, hypoglycaemia, pulmonary oedema, circulatory collapse, cerebral disease, acidosis and spontaneous bleeding (Bag et al., 1994).

The severity of the ailment can be ascertained by the extent of hepatic and renal dysfunction. In addition, renal tubular changes relative to glomerular changes are more prevalent with *Plasmodium falciparum* infection which concomitantly leads to complications like acute renal tubular necrosis and acute renal failure associated with oliguria and hypercatabolism (Gomes et al., 2011). Malaria infection in endemic areas has been reported to contribute to acute renal failure among children (Mockenhaupt et al., 2004), and is accompanied by low specific gravity in urine, metabolic acidosis and a rise in serum urea, potassium and sodium (Padhi and Mishra, 2012). Thus, a sharp increase of creatinine and urea could be a reliable indicator in assessing renal malfunction among malaria-infected people (Ebele et al., 2010; Jasani et al., 2012). Acute renal failure in malaria has been defined by WHO as creatinine > 265.2 µmol/l with urine output < 0.5ml/kg/hr, irrespective of patients being rehydrated, and with varying forms of asexual
formation of *Plasmodium falciparum* found in their peripheral blood film (Goyal *et al.*, 2016). Varied etiological factors augment the advancement of acute renal failure in malaria: volume depletion accounting for (51%), intravascular hemolysis (39%), cholestatic jaundice (33%), hypotension (30%), disseminated intravascular coagulopathy (DIC) (7.4%), and sepsis (9.6%) (Kumar, 2002).

According to Onyesom and Onyemakonor (2011), high levels of liver enzymes in serum following malaria infection in patients are good indicators in assessing liver malfunction. The alterations in hepatic cells caused by the sporozoites may contribute to the release of parenchymal and membraneous enzymes into the microcirculation leading to the rise of liver enzymes (Burtis *et al.*, 2001).

Jaundice in malaria, especially *falciparum* malaria depicts severe ailment that presents with more advanced complications and poor diagnosis (Murthy, 1998). Jaundice that occurs in severe falciparum malaria has been reported as multifarious which are connected with parasitized hemolysis of red blood cells and non-parasitized hemolysis, hepatic malfunction and disseminated intravascular coagulation (Bruneel *et al.*, 2003). These are likely to be present separately or in the combination of other complications and could alternate from mild to severe (Bruneel *et al.*, 2003).

For several years now, severe malaria manifestations have been predominantly of cerebral origin (Idro *et al.*, 2010), but recently, the linkage with hepatic dysfunction and renal failure, although somewhat established in adults, is less known among children under five years. There is basically few data at hand on the predominance of hepatic and renal dysfunction in malaria infected children less than five years in Ghana. This study thus seeks to evaluate hepatic and renal dysfunction among children less than five years with malaria infection in Jasikan district, Oti region– Ghana.
1.1 Problem Statement

Malaria is the leading cause of mortality and morbidity in children in Ghana (UNICEF, 2011; Afoakwah et al., 2018). According to the National Malaria Control Programme (2015), the disease kills a minimum of three (3) children daily and is the highest among out-patient department (OPD) cases in Ghana. In a research by Singleton and Osei (2014), the disease accounts for low productivity, poverty and high financial burden to households and negatively affects the economy of the country. A high malaria incidence in a country has a direct negative impact on the countries average per capita Gross Domestic Product relative to a country without malaria (Sachs, 2002).

In Africa alone, the disease is estimated to cost the continent US$12 billion annually (Greenwood, 2005). The high financial impact constitutes reduced missed working days due to the ailment, absenteeism from school, and loss of tourism attraction (Greenwood, 2005).

Malaria infection can lead to acute renal failure; mechanical obstruction by infected erythrocytes, immune-mediated glomerular deposits, and fluid loss (Barsoum, 2000). Further to this, malaria-induced jaundice, shock, severe anaemia, high concentration of plasma creatinine, low urine output and delayed to the hospital are associated with the high mortality rate (Sheiban, 1999). Further, the current standard of the management of acute renal failure: the high-quality intensive care and prompt institution of renal replacement therapy are highly expensive and a financial burden to many families accounting for high mortality rate. Again, liver changes that ensue during malaria infection have been characterised with hyperplastic kupffer cells, portal tract inflammation, cholestasis and liver necrosis (Viriyavejakul et al., 2014). These changes in the kidney and liver could lead to renal and liver dysfunction.
1.2 Justification of the study

The mortality and morbidity rates of malaria in children are relatively high in Ghana (Nonvignon et al., 2016; Afoakwa et al., 2018) and hence more knowledge is needed to manage malaria infection among children under five years. There is however, little information related to the incidence of malaria infection and its involvement in renal and hepatic dysfunction among children less than 5 years. In Ghana, assessment of kidney and liver function among children suspected with malaria infection is, however not routinely done.

Malaria infection in children may lead to severe complications and even death, including acute renal failure, jaundice, haemoglobinuria, circulatory collapse, and cerebral malaria (Conroy et al., 2016). It is thus, imperative to evaluate the renal and hepatic malfunction in malaria cases among children to mitigate malaria-mediated complications (Ogbadoyi & Tsado, 2009).

Diverse research highlights the effects of malaria on the liver and kidney among adults in many endemic countries (Sharma et al., 2004; Ogbadoyi & Gabi, 2007), but less research has been conducted in under five children in that regard. It is against this backdrop that this study seeks to assess the malaria involvement in kidney and liver dysfunction among under-five year children in the Jasikan District, Oti-region, Ghana. In addition to providing baseline data, this study will help in instituting proper and efficient management systems in reducing the adverse effects of malaria among children.
1.3 Null hypothesis

Malaria infection has no effect on both the kidney and liver in children less than five years.

1.4 Aim of the study

The aim of this study was to evaluate the effects of malaria infection on renal and hepatic function among children less than five years in Jasikan District, Oti-region of Ghana.

1.5 Specific objectives

- To determine renal (urea, creatinine) and liver (AST, ALT, ALP, bilirubin, total protein and albumin) biochemical markers in malaria and non-malaria patients.

- To determine associations between degree of parasitaemia with liver and kidney function tests as markers of organ dysfunction.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of malaria

Malaria acquired its name from the Medieval Italian expressly “mala aria” meaning “bad air”. The ailment was previously described as ague fever or Marsh fever as a result of its correlation with the wetland covered with vegetation (Reiter, 2000). Even though the disease was characterized years ago, it was a French medical doctor, Charles Louis Alphonse Laveran who described the causative agent as a protozoan belonging to the genus Plasmodium (Barsoum, 2000). The Plasmodium, the causative agent of malaria, is extensively established in numerous tropical countries globally including South and Central Africa, sub-Saharan Africa, and Southeast Asia. The disease is thought to be prevalent in such regions due to the very high humid and hot climatic conditions creating a natural habitat for the growth of the ailment vector, Anopheles mosquito (Elsheikha & Sheashaa, 2007). There are five (5) main species of the genus Plasmodium that cause malaria among humans: *P. vivax*, *P. malarae*, *P. ovale*, *P. knowlesi* and *P. falciparum*. Among the above-listed species, the most lethal is the *P. falciparum* and *P. vivax* (WHO, 2018). According to WHO (2017), *P. falciparum* accounted for 99.7% predominant causes of malaria in sub-Saharan Africa, while *P. vivax* accounted for 74.1% of the majority of malaria cases in America. The mechanism by which the parasites spread to humans is by the bite of the vector infected female Anopheles mosquito. Generally, there are about four hundred (400) species of Anopheles mosquito identified; approximately thirty (30) species are of considerable significance (WHO, 2018), amongst all the relevant species, *Anopheles gambiae complex* and *Anopheles funestus* are the most prevalent. In humans, the five Plasmodium species that poses grave threats has various clinical symptoms, geographical distribution, complications, and relapse fashion. The
infection could be mixed or a mono infection (Coban et al., 2018). The predominant
species in sub-Saharan Africa, the *Plasmodium falciparum*, contributes to most of the
severe cases and complications related to malaria in the region including, severe anaemia,
cerebral malaria, respiratory distress (Coban et al., 2018). It is the predominant cause of
death in children in sub-Saharan Africa and accounts for 24% of mortality cases among
children in sub-Saharan Africa (Murray et al., 2012). By comparison, *P. vivax* is the most
lethal outside Africa. It has been stated by Naing et al., (2014) that *P. vivax* is the most
underestimated cause of death in the world though contributing to high morbidities
around the globe. It is not a contributing factor for the high incidence and mortality rate
of malaria in the central and west Africa since a measurable number of the population are
Duffy-negative, which connotes that erythrocytic invasion by the parasite is obstructed.
In other tropical regions in the world the *P. vivax* are in synchronism with other
*Plasmodium* species hence the prevalence of a mixed infection (Crowley, 2010).

However, in regions where *P. vivax* is prevalent transmission rates have been low
contributing to the population acquiring low immunity to the parasite hence presenting a
high risk of infection among all ages but much severe in children (Crowley, 2010). In
current times, it has been noted that *P. knowlesi* a similar parasite has become a
significant cause of illness in southeast Asia predominantly a zoonosis (Ahmed and Cox-
Singh, 2015) but further evidence proves to the fact that it can be transmitted to humans
and contribute to devastating illness including kidney failure and acute lung diseases
(Millar & Cox-Singh, 2015). *Plasmodium ovale*, a parasitic protozoan that causes tertian
malaria among humans, has been shown to consist of subspecies such as *P. ovale
walliker* and *P. ovale curtisi* and more restricted in its territory to the Philippines, West
Africa, Eastern Indonesia and Papua New Guinea (Sutherland et al., 2010; Faye et al.,
1998). The reported prevalence rate of *P. ovale* in West Africa has been ten percent
(10%) with estimated morbidity to be fifteen million cases annually (Sutherland et al., 2010). *Plasmodium malarae*, on the other hand, causes quartan malaria and has been noted to account for milder clinical symptoms in humans relative to the other species such as *P. falciparum* and *P. vivax*. *Plasmodium malarae* is widely distributed in sub-Saharan Africa, South America, and Indonesia with a prevalence proportion in the endemic regions as between 4% and 20%. *Plasmodium malarae* is reported as the least researched of the five species that infect man and highly underreported (Bruce et al., 2007; Mohapatra et al., 2008).

2.2 Epidemiology and Global Burden of Malaria

Malaria continues to be the major cause of morbidity in the world. The disease was approximated in 2011 to infect about 3.3 billion people globally, of which sub-Saharan African regions were considered the worst affected (WHO, 2013). In 2015, WHO globally predicted two hundred and fourteen (214) million new cases of malaria that culminated into 438,000 mortalities globally (WHO, 2015). Olupot and Maitland (2013), predicted the number of cases of malaria to be in the range of three hundred and fifty (350) and five hundred and fifty (550) million with regards to falciparum malaria. The ailment resulted in a range of 655,000 and 1,240,000 mortalities in 2010 (Murray et al., 2012). A proportion of 92% of malaria cases and 93% of mortalities occurred in the sub-Saharan region, where it continues to be the predominant cause of death and severe complications among children and pregnant women; however, an approximated proportion of 61% of malaria mortalities were among children less than five (5) years of age (WHO, 2018). The high incidence of morbidity and mortality among children between six (6) months and five (5) years of age could be attributed to the low maternal immunity and the under-developed child’s subsequent immunity against the infection. An estimated number of one hundred and five (125) million pregnant women worldwide
were at danger of malaria infection annually; however, in the sub-Saharan African region, malaria in pregnancy accounted to two hundred thousand (200,000) of infant’s deaths annually (Hartman et al., 2010).

The incidence of malaria cases in Western Europe was around 10,000 and that of the United States within 1300-1500. An approximated number of 900 mortalities were recorded in Europe within the ten year period of 1993 and 2003. The burden of the ailment, in recent years the global incidence of malaria and its ramifications have declined by 60% in 2015 from a figure of 985,000 deaths estimated in 2000 (WHO, 2015), the reduction of mortalities have been as a result of the extensive use of artemisinin-based combination therapy and the insecticide-treated nets (Howitt et al., 2012).

Malaria is predominant in tropical and subtropical areas due to the high temperatures, rainfall and high humidity as well as the stagnant waters becoming conducive for the development of the mosquito larvae. The disease is thought to be more pronounced in rural settings relative to the cities (Cui et al., 2012). However, Machault et al., (2011) attest that the disease in Africa is more evident in both cities and rural areas. The disease condition incidentally has been in coexistence with more significant implications on the economy of countries where the ailment have been predominant. According to Humphreys (2001) in the 19th and 20th centuries, the disease contributed to a significant negative impact on the growth of Southern American states.

Generally, countries with the high impact of malaria, the disease accounts for 50% of outpatient department (OPD) visits, 30-50% of admissions of hospitals and an estimated figure of 40% of the national expenditure on public health (WHO, 2003). In Africa, the disease is one of the predominant causes of neurological dysfunctions among school
children leading to reduced cognitive capabilities and low academic performance even after recuperation (Fernando et al., 2010).

2.3 Malaria in Ghana

In Ghana, malaria is a public health threat with *Plasmodium falciparum* as the prevalent species causing the ailment. The disease has consistently been the prominent cause of morbidity and mortality among children less than five years and accounts for 44% of *falciparum malaria* cases in the out-patient department of hospitals (Bahaah et al., 2019). The disease negatively affects productivity, economic development of households and the economy of the country (Thuilliez et al., 2017; Singleton and Osei, 2014). The ailment is the primary cause of child absenteeism in schools in the state. During the year (2006) under review, UNICEF Ghana attested that from a reported figure of 3.5 million morbidity cases of suspected malaria in the public hospitals in the country, about 20,000 children less than five years predominate. Hence, the disease chronicled sixty-one (61) per cent of admitted cases in the hospitals and twenty-five (25) per cent of deaths among children less than five years (UNICEF Ghana, 2007). The National Malaria Control Programme asserted the preponderance of the disease in almost all Out-Patient Department (OPD) morbidities and mortalities in children and the ailment account for three deaths in children daily in the country (NMCP, 2016). In a concerted efforts by various countries to reduce and interrupt the incidence of malaria by the year 2015; Ghana initiated some control mechanism to achieve the desired goal, such as the increase of long-lasting insecticide nets coverage and usage by 80% and 60% respectively by 2010. Some of the measures include prompt diagnosis and therapy management with Artemisinin-based combination therapies (ACT’s) and increase the scope of intermittent preventive treatment (IPT) among pregnant women. Even though the methods mentioned above in the country influenced the reduction of malaria morbidities among children,
there are nonetheless a high number of children less than five years afflicted with the disease in the country (Appiah et al., 2017). According to Appiah et al., the condition is still hyper-endemic in the country which has a predominant range of 11.2% - 40 %. The prevalence rates of malaria in children residing in the rural communities are higher relative to the children living in the urban areas, which confirm the study by Multiple Indicator Cluster Survey (MICS) in 2011. The MICS study showed that children less than five years with malaria in the urban cities have a lesser prevalence rate of the disease in comparison with the other children in the rural communities: 14% and 44% respectively (NMCP, 2013).

2.4 Life Cycle of Malaria Parasite
2.4.1 Pre-Erythrocytic stage

Following the deposition of sporozoites from the salivary glands of the female Anopheles mosquito into the vascular tissue of the skin during blood meal probing, malaria infection ensues. In about a minute interval, the sporozoites migrate rapidly through the capillary wall and then invade the bloodstream (Lamb, 2012). Phagocytes destroy most of the sporozoites inoculated into the bloodstream while others move out of the vascular system approximately forty (40) minutes and enter the hepatocytes of the liver to undergo the pre-erythrocytic schizogony (John and Petri, 2006). This process takes 7-10 days with no symptoms.

As the sporozoites reach the liver, it then moves through the sinusoidal epithelium in the organ negotiating through various resident liver macrophage (kupffer cells), space of disse and finally invading a hepatocyte, hence the formation of a parasitophorous vacuole (pv) (Lamb, 2012). This then undergoes a polymorphous nuclear division process which then leads to cytoplasmic division called the schizogony (Greenwood et al, 2012). The hepatocyte that is infected then develops into a bigger exo-erythrocytic shape called the
schizont. This then develops into 10,000 and 20,000 merozoites over a period of 7-10 days. However, with respect to *P. vivax* and *P. ovale* their development in the liver into schizont is delayed in the hepatocytes and remain dormant as hypnozoites. Relapsing of malaria infections is principally caused by *P. vivax* and *P. ovale*, which are mainly anti-malaria drug-resistant (Lamb, 2012).

### 2.4.2 Asexual Erythrocytic cycle

As merozoites rupture from the hepatocytes, they traverse into red blood cells (RBC) and then develop through the asexual erythrocytic cycle. The infiltration of merozoites into the new RBCs comprises of the release of proteases from the anatomical structures located at the apex of the merozoites termed as micronemes, dense granules and rhoptries. However, the dominant surface proteins of merozoites are named as merozoite surface protein (MSP)-1 (Lamb, 2012). The significance of this protein is paramount for asexual cycling within the erythrocytes stages; it is proteolytically refined during several phases after it reaches the surface of the merozoite, which is an essential process for invasion. On the other hand, *P. vivax*, which depends upon the existence of a glycoprotein located on the red blood cells (RBC) surface, termed the Duffy antigen a prerequisite for attachment (Lamb, 2012). This specie (*P. vivax*) is not the leading cause of malaria burden in sub-Saharan Africa, and this is because the population is of Duffy-negative blood type. Individuals with Duffy-negative blood type have RBCs resistant to the infiltration of *P. vivax*. Furthermore, because RBCs lack nuclei and are also metabolically dormant cells, growing parasites obtain amino acids that are needed by digesting haemoglobin (Lamb, 2012).

During schizogony stage, the moment the infected red blood cells are ruptured or lysed, it releases merozoites, hemozoin and other metabolic wastes into the bloodstream (Lamp,
The lysed parasitised RBC’s are coordinated in a manner that the rupture of the cells occurs at a defined space of time typical for each species. The above statement explains the sessions of fever that occurs as a result of the released metabolic wastes (Brown et al., 2019). The merozoites released are within the range of 10-32, and these eventually pervade into new RBCs to activate a new erythrocytic phase (Lamb, 2012).

2.4.3 Transmission in Mosquito

A minimal amount of infected red blood cells separate into a male and female gametocytes, although the precise molecular mechanism that develops into male and female gametocyte (mature sexual cells) are unknown (Lamb, 2012). Within the mosquito’s stomach, the pH alteration and the temperature variation stimulates cell division and differentiation in the formation of gametes and fertilisation resulting to the creation of mobile paired (diploid) ookinetes (zygote) that leaves the bolus of the blood meal and migrate through the midgut epithelium to become sessile oocysts (Lamb, 2012). In a duration that spans between 10-14 days, sporozoites then grow in the oocyst through mitosis, and these traverse through an enzymatic process into the body cavity of the mosquito. The sporozoites then migrate through the haemolymph and adhere to the basal membrane of the mosquito salivary glands, accessible for injection into a man or the subsequent host (Lamb, 2012).
2.5 Clinical Manifestations of Malaria

The severity and development of a clinical encounter of malaria rely on the type of species and strain of the Plasmodium parasite, immune capacity, genetic status, age, mode of transmission, prophylaxis intake, and antimalarial drug exposure. The febrile malaria illness emerges from the lysis of red blood cells that are parasitised with subsequent deposits of merozoites into the vasculature at the end of the asexual reproduction. The alternating febrile primary attacks are perceived to be characteristic of Plasmodium species causing malaria; however, such phase of attack could manifest for some days before being established and could be altered by earlier immunity. If the symptoms are treated with suitable drugs, the symptoms settle over some few days, even though with fatigue and feebleness. With *P. falciparum*, a full-fledged treatment would eliminate the disease while a reappearance of the symptoms depicts a poor treatment, or a
re-infection, or a resistance to drugs (Collins and Jeffery, 1999). By contrast \textit{P. ovale} and \textit{P. vivax}, further reoccurrence of infection ensues by the recrudescence of the hypnozoite in the liver, except that the hypnozoite stage is cleared by an 8-aminoquinoline medication (Collins and Jeffery, 1999). Patients that suffered from previous malaria infection and acquired a partial immune status, the merozoite release by the schizonts that induced febrile episodes accompanied with almost forty eight (48) hours for \textit{P. ovale} and \textit{P. vivax} whilst \textit{P.falciparum} and \textit{P. malariae} infections occurs in each seventy-two (72) hours in an infrequent febrile illness, basically during the first days of the ailment (Agrawal and Teach, 2006). For children, manifestations of the disease are diverse, which mostly imitates some common childhood ailments. High temperature and migraine may sometimes be the main symptoms or gastroenteritis could be the signs of illness prevailing. High temperature (>40°C) is the main symptom in children with the typical quartan and tertian pattern perceived in less than 25% of children and could lead to convulsions. Symptoms such as vomiting and nausea are most prevalent with \textit{P. falciparum} infection and could likely inhibit oral anti-malarial drugs treatment (Shingadai \textit{et al}, 2011). Among the predominant comorbid conditions related to malaria, pneumonia and acute diarrhoea are a distinct determinant cause of mortality in children. Most children with malaria and pneumonia also have a coexisting viral and bacterial characteristic. Similarly, sudden diarrhoea could be characteristic of clinical malaria or rather the comorbid of diarrheal illness originating from enteric microbes. Most children are more likely to have a unique feature related to \textit{P. vivax} as hepatomegaly, jaundice, and splenomegaly relative to adults with chills, pains in the muscles and headache (Opoka \textit{et al}, 2008).

Severe complications in malaria are more prevalent with \textit{P.falciparum} since \textit{P.falciparum} infect all stages of erythrocytes and leads to high parasitaemia (60%) (Schumacher &
Over the years, several studies have endeavoured to analyze how malaria infection could lead to severe disease in some people but still prevail in others as asymptomatic (Billing et al., 2012). Most complications in malaria result from vascular endothelium obstruction leading to tissue ischemia, and hemolytic anaemia; some of the characteristic features of complicated malaria involve high aminotransferases, respiratory stress, acidosis (pH<7.3), hypoglycaemia (<2.2 mmol/l), severe anaemia (Hb <5g/dl) and high levels of parasitaemia (that is 5-10% of red blood cells infected) (Ladhani et al., 2002; Modiano et al., 2001).

2.6 Congenital Malaria

Expectant mothers are more prone to be infected by Plasmodium parasites and are, to a greater extent inclined to develop detrimental outcomes, especially among first time pregnant women and their babies. Apart from the mother being infected with the disease, malaria can also affect the fetus, and placenta which advances to the birth weight of the child (Low birth weight), and predevelopment (Murphy and Breman, 2001). The approximated figure of low birth weight associated with malaria is within the spectrum of 7.8-45.3 per 1000 live births with its related risks at the initial (first) month of life is estimated to be forty (40) times relative to healthy birth weight of babies (Murphy and Breman, 2001). The disease can be transmitted congenitally but has not been seen periodically as a result of the effective blockade activity of the placenta. Even though in the endemic regions congenital malaria develops in 0.1% among the immune and 10% among the non-immune women, the occurrence of placental infection is estimated in one-third of pregnant mothers. However, in endemic regions, to differentiate between malaria transmitted to infant during pregnancy and that transmitted to infant during post-natal from mosquitoes has been challenging.
The disease incipient symptoms are insidious and mostly ensues two (2) to eight (8) weeks of age with the usual febrile ailment absent, while the child rather manifest symptoms similar to sepsis (Del Punta et al., 2010) such as vomiting, poor feeding, diarrhoea and irritability. Occasionally hepatosplenomegal and fever may be presenting on examination. The most predominant laboratory findings include anaemia, hyperbilirubinemia, and thrombocytopenia (Schumacher and Spinelli, 2012). In consecutive pregnancies, most women build up antibodies aimed at the *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) strains required in chondroitin sulfate A (CSA) mediated cell adherence (Ataide et al., 2014). Moreover, with regards to treatment, infants do not require treatment associated with the exo-erythrocytic phase of the parasite relative to their mothers; however, some studies propound that a smaller number of infants that directly got affected by the malaria infection were at higher danger of later life infections (Hartman et al., 2010).

### 2.7 Pathogenesis of Severe *P. falciparum* Malaria

The *P. falciparum* characteristics that lead to the development of the ailment and consequently death include microvascular obstruction, high parasitaemia, stimulating host inflammatory responses and the activation of the endothelium (Wassmer et al., 2015). The high parasitaemia with a ten (10) fold growth at each forty-eight (48) hours indicates that an increase in the entire body parasitaemia is attained rapidly. The significance of the above statement disguised the development of the segregation of the parasite binding to the blood vessels lining hence parasite density determined by microscopy is underrated (Hendriksen et al., 2012). The approximation of biomass established on the parasite's protein such as the histidine-rich protein (HRP) depicts a clear understanding of the parasite load (Hendriksen et al., 2012). The high increase of
the parasite development activates an immediate inflammatory action. According to Cunnington et al. (2013), he argued that severe malaria cases mostly develop as a result of excessive or badly curbed responses generated to restrain the acute transmission. Again, the high severity of malaria and its outcome as death are associated to pro-inflammatory cytokines in several studies (Kwiatkowski et al., 1990) as well as the levels of IL-1B known to be prognostic of childhood severity of malaria. However, a differentiated pro-inflammatory reaction is likely the contributory factor to the pathological process involved in severe malaria differently, meaning both topical and general effects (Cunnington et al., 2013).

2.8 Microvascular Obstruction

The fundamental characteristic of severe malaria on account of *P. falciparum* is primarily the obstruction of the vascular endothelium due to the segregation of developed parasites in the small vessels of the vascular system (Dondrop et al., 2008). This leads to metabolic acidosis as well as topical end organ impairment and to the highest degree in the brain. Further implications of the segregation of parasites and the accompanying impairment of the flow of blood have been discovered in tissue beds such as the retina and rectal mucosa (Dondrop et al., 2008).

The sequestration of parasites ensues due to the alteration of the erythrocyte surface to adhere to receptors on several cells (Smith et al., 2013). The cytoadherence which leads to seclusion of parasitized erythrocytes in capillaries results in obstruction of the microcirculation and low oxygen in tissues (Silamut et al., 1999). Furthermore, the microcirculation flow is hypothesized to be compounded as a result of the inflexibility of both the uninfected and infected erythrocytes as well as the clumping of the infected
erythrocytes and the non-parasitized erythrocytes attaching to parasitized red blood cells in a development known as resetting (Dondrop et al., 2004).

2.9 Renal involvement of Malaria

Malaria infection was the foremost of parasitic transmission to be lucidly connected to glomerular diseases in Sub-Saharan Africa (Elsheikha & Sheashaa, 2007). Renal disease associated with malaria is basically as a result of red blood cells malformation. In this case, red blood cells that are parasitised are inclined to stick to healthy red blood cells, thrombocytes and capillary endothelium. The phenomenon progresses to the development of rosettes and clumps, and subsequently impedes the distribution of blood in the smallest blood vessel (Barsoum, 2000). These occurrences are presumably partly responsible for kidney injury, in relation with instability in the dynamics of blood flow, including volume depletion of blood plasma and shock. The inducement of the endothelium advance to the liberation of varied cytokines, together with catecholamines, endothelin, thromboxane as well as several inflammatory mediators implicated in the development of kidney injury associated with malaria (Silva, 2017).

The activation of the natural defense system in malaria uses the T helper -1 (Th1) and T helper-2 (Th2) response. The activation of Th2 response during *P. malariae* infection leads to the stimulation of complement with immune complex deposits progressing to glomerulonephritis. Again, the hemodynamic irregularities as a result of increased parasitised red blood cells advance to acute tubular necrosis, a feature of *P. falciparum*. However, when Th1 is activated, sudden glomerulonephritis and sudden interstitial nephritis become apparent (Silva, 2017). According to Naqvi (2015), cortical necrosis has been elucidated in malaria as a feature of a more severe kidney injury and generally connected with renal non-recovery function and eventually end-stage kidney disease.
These complications are attributed to varied factors: volume depletion of blood plasma, constriction of blood vessels, lysis erythrocytes (leading to hemoglobinuria), parasitized red blood cells, immune complexes deposition in glomeruli, circulation of blood in blood vessels impairment (a results of cytoadherence of parasitised erythrocytes) and rhabdomyolysis (though not a common feature in malaria) (Suravu et al., 2014; Koopmans et al., 2015; Mohapatra et al., 2016).

Further contributory component for renal disease in malaria include hepatic malformation, as well as hepatomegaly and jaundice, by which high levels of bilirubinemia could lead to acute kidney injury (hepato-renal syndrome) (Kute et al., 2012; Saravu et al., 2014; Gomes et al., 2011). Furthermore, acute kidney injury has been reported in several diverse Plasmodium species including P. falciparum, P. vivax, P. malariae and P. ovale which tends to exacerbates as a result of reduced hydration and fluid loss induced by vomiting, sweating and feverishness. Studies on histology depict glomerulonephritis, sudden tubular necrosis and interstitial inflammation of the kidney. It is reported that malaria infection among patients with repeated episodes is principally associated with chronic renal disease (Naqvi, 2015; Barsoum, 2015; Siriwardhana et al., 2015).

2.9.1 Renal involvement by P. falciparum

Acute kidney injury is recognized as one of the outcome of malaria which accounts for 40% of patients suffering from a severe state of P. falciparum in sub-Saharan Africa, with an eminent mortality rate at a figure of 75% of clinical cases (Koopmans et al., 2015). It has been elucidated that P. falciparum is the principal cause of severe forms of malaria and also a predominant agent responsible for the majority of acute kidney injury (Win, 2012). According to Naqvi et al., (2016) and Win (2012), clinical manifestations of
Plasmodium falciparum infections involve oligo-anuria accounting for 46-76%, high catabolic state, and severe metabolic acidosis. Most of the acute kidney injury that occurs in severe malaria, renal involvement has been one of the principal elements for categorizing patients with severe P. falciparum malaria (WHO, 2015). Some of the high risk factors associated with acute kidney injury in malaria include: late referral from another hospital, jaundice, oligo-anuria, high levels of white blood cells, changed consciousness state and P. falciparum infection relative to P. vivax infection (Win, 2012). Again, electrolytes dysfunction that ensues during malaria connected with acute kidney injury includes low levels of sodium (hyponatraemia) accounting for 30-50% of patients (Barsoum, 2000; Naqvi et al., 2016), and high levels of potassium (hyperkalaemia). The primary mechanism involved in acute kidney injury with regards to malaria has not been wholly appreciated; however there are few hypotheses that have been explained including impairment of the kidney’s microcirculation, immune-mediated glomerular injury and hypovolemia (Koopmans, 2015). According to Koopmans (2015), acute tubular necrosis is the cardinal renal findings in malaria-related severe kidney injury, with less prominent, glomerulonephritis (more common in children). It is reported that about 20-50% of malaria-associated acute kidney injury also presents with mild urinary proteins (proteinuria), mild albumin in the urine (albuminuria) as well as the presence of casts in urine (Elsheika & Sheashaa, 2007). In a research study by Elsheika and Sheashaa (2007), they reported of the fact that, even though the connection between P. falciparum infection and nephrotic syndrome is quite rare, the association between P. falciparum and oedema, cast nephropathy, acute tubular necrosis and inflammatory interstitial pervasion has been moderate. New biomarkers concerning kidney injury have recently been studied in malaria caused by P. falciparum: kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL), these markers are advantageous over the
orthodox markers: creatinine (van Wolswinkel et al., 2016). In an article by van Wolswinkel et al. (2016) they noted that about 31% of participants infected with malaria and its associated acute kidney injury had creatinine at normal levels explaining the importance of the novel biomarkers of severe kidney injury.

Malaria infection leads to immune compromised state due to the up regulation of macrophages with subsequent depletion of macrophages. The high production of cytokines, and depletion of T cells, which is induced when the organism reaches the innate immune system leads to the stimulation of the B cells with the production of immunoglobulins (Basoum, 2000).

Scientists are still discussing the players involved in the molecular mechanisms of malaria nephropathy, and it has been indicated that there is the interaction of IL-10, IL-1α, IL-6, colony–stimulating factor (GM-CSF) and TNF-α (Barsoum, 2000; Elshekha & Sheashaa, 2007). There have been several studies that demonstrate the existence of lesions in the glomerulus as a result of *P. falciparum* infection; the injury is typified with the dominant mesangial increment along with episodic thickening of the basal membrane, Bowman’s capsule and the mesangium (Das, 2008). Furthermore, Das (2008) indicated the predominant characteristic of malaria *P. falciparum* infection to be interstitial nephritis relative to nephropathy, which is described as the outcome of the patient’s immune reaction to the parasitic infection.

### 2.9.2 Management of Acute Kidney Injury in Malaria

Malaria-related renal disease treatment encompasses fluid replacement, dialysis, and suitable antimalarial drugs (Kute et al., 2012; Plewes et al., 2015). According to Maheshwari et al. (2004), suitable use of antimalarial drugs and dialysis assistance was required for thirty-five (35) patients out of the eighty one (81) *P. falciparum* malaria
cases studied. Maheshwari et al., (2004) reported further that 18.5% of the patients that died as a result of multiple organs failing due to malaria acute renal failure (MARF). In a separate investigation, the possibility of a kidney substitute therapy for malaria acute renal failure generally depicts a better outcome (Prakash et al., 1996). In the treatment of oliguric and nonoliguric acute renal failure associated with malaria, hemodialysis was considered highly beneficial when initiated promptly during the ailment of the patient (Wilairatana et al., 1999). Another study by Naqvi et al., (2003), evaluated about two thousand nine hundred and eight (2,098) subjects infected with acute renal failure, out of the total subjects, one hundred and twenty four (124) had malaria acute renal failure (MARF) due to *P. falciparum* (121) and *P. vivax* (3). Again, ninety-nine (99) subjects needed renal substitute therapy. However, from the total number of one hundred and twenty four (124), seventy-seven (77) subjects representing sixty-two (62%) per cent of patients recovered completely from renal replacement. The author concluded that prompt referral to standard hospitals was essential in providing requisite therapies such as antimalarial and dialysis support to patients, hence mitigating mortalities and increase renal function recovery.

The hypercatabolic condition of malaria-associated acute renal failure due to *P. falciparum* requires imperative hemodialysis and peritoneal dialysis during an elevated creatinine level or concentration. However, hemodialysis has been discovered to be more effective relative to peritoneal dialysis due to the obstructed peritoneal circulation of blood within the microvessels (Barsoum, 2000; Phu et al., 2002; Eiam-Ong, 2003; Trang et al., 1992).
2.10 Hepatic involvement of Malaria

Liver involvement in *P. falciparum* malaria infection is a common phenomenon, and the existence of jaundice (serum bilirubin > 3mg/dl) depicts a severe or complicated case of malaria infection (WHO, 2000). According to WHO (2000), with the exception of jaundice, other manifestations of hepatic malfunction have been rare. Recent studies on malaria hepatopathy have been increasing precisely from several Asian countries, particularly India (WHO, 2000). The preponderance of the infection is due to *P. falciparum* and occasionally from a mixed infection: *P. falciparum* and *P. vivax* (Joshi *et al.*, 1986). The range of renal connection concerning malaria alternate from the slightest of liver function tests abnormalities to liver encephalopathy (Ahsan *et al.*, 1993).

2.10.1 Malarial Jaundice

The prevalence of jaundice in malaria among patients with severe malarial infection is known to be 2.58% (Anand and Puri, 2005). Jaundice may be as a result of severe lysis of red blood cells or liver involvement. The breakdown of the hepatocytes during the initial schizogony culminates into cellular injury but rarely is it cardinal in liver malfunction (Anand and Puri, 2005). High levels of *P. falciparum* infested in erythrocytes with its concomitant lysis of the red blood cells leads to the increase of serum bilirubin, predominantly unconjugated bilirubin. The malaria parasite, *P. falciparum* infecting a large number of erythrocytes is further broken down by the spleen with the aid of macrophages results in hemolytic anaemia. The segregation of the parasitised erythrocytes in capillaries leads to impairment of blood vessels and blood deficiency in cardinal organs like the liver resulting in hepatic dysfunction (Bhalla *et al.*, 2006).

Malaria hepatitis is a terminology used to expound liver malfunction in complicated malaria; however, an absolute inflammation of the hepatocytes is less common (Anand
The term malaria hepatitis is typified with an elevated bilirubin with an increase in aminotransferase levels by three times relative to the standard upper limit (Anand et al., 1992); and the above statement is in accordance with the nonappearance of exposure to harmful drugs to the liver and viral hepatitis (Bhalla et al., 2006).

Most literature on malaria effects on the liver has comprehensively depicted how *P. falciparum* malaria have had high adverse impact on the liver. Similarly, other research have reported of mixed infection including *P. vivax* (Joshi et al., 1986) and hepatitis E as well as *P. falciparum* leading to hepatitis of malaria origin (Bansal et al., 2002). The prevalence of hepatitis and jaundice among people infected with malaria due to *P. falciparum* infection has been irregular or fluctuating. A research analysis involving seven hundred and thirty-two (732) adults subjects infected with *P. falciparum* malaria, a minimal number (39) of patients were classified as jaundiced, while eighteen (18) of the subjects were indicated to have had malaria hepatitis (Anand et al., 1992). In a separate article, a sample size 95 admitted subjects with malaria due to *P. falciparum*, resulted in 62% with jaundice and twenty-one per cent (21%) with hepatitis of malaria origin (Murthy et al., 1998). Another study conducted in Poland among one hundred and twenty-one (121) malaria-infected subjects, a total percentage of thirty-seven (37%) manifested a dysfunction of the parenchymal cells of hepatic origin (Goljan et al., 2000).

Among adults, high levels of bilirubin in serum prevalence rate as a result of malaria infection (severe) ranges from 32-37%. The preponderance of the cases constitutes mostly of unconjugated hyperbilirubinemia (Harris et al., 2001). The disease (malaria hepatitis) is predominant in children with severe falciparum malaria, with an occurrence ranging from 8% to 32% (Satpathy et al., 2004). The differences in the account of liver cellular malfunction and jaundice could be dependent on the age group investigated,
malaria endemicity region, geographical setting, comorbid viral hepatitis as well as helminth infections (Anand & Puri, 2005).

2.10.2 Pathophysiology of Hepatic Involvement of *P. falciparum* infection

*Plasmodium falciparum* tends to infiltrate all types of erythrocytes of varying ages, hence encouraging the development of high parasitaemia or severe ailment. The possibility of acute disease is predominant among children less than five years and pregnant women. The active breakdown of a higher number of parasitised erythrocytes by macrophages in the spleen and the subsequent release of merozoites inclines to proceed to hyperbilirubinemia and severe anaemia (Autino *et al.*, 2012)

The *P. falciparum* has a particular trait; such that the parasites grow within erythrocytes and then stimulate the development of adhesive knobs to present superficially on red blood cells (Newbold *et al.*, 1999; Oh *et al.*, 1997). The sticky knobs then attach to the receptors located on the endothelial cells in capillary vessels. The sequestration and cytoadherence of the erythrocytes within capillaries contributes to the impairment of the flow of blood in the microvasculature. Furthermore, the parasitised red blood cells adhere to the unparasitised red blood cells leading to the formation of clumps and rosettes with subsequent impairment in the microcirculation. Finally, hepatocytes malfunction ensues due to the development of ischemia as a result of the circulation of blood in minute blood vessels modifications and further lead to several complications depicting as organ malfunction in the individual (Bhalla *et al.*, 2006).

The hepatocellular dysfunction ensues as a result of the following: micro occlusion of the portal venous due to infected erythrocytes (Molyneux *et al.*, 1989), cholestasis of intrahepatic origin due to the blockage of the microvasculature and subsequent interference of the hepatic microvilli (Autino *et al.*, 2012), endotoxemia as a result of the
severe malaria infection (Bhalla et al., 2006), Kupffer cells hyperplasia as a result of haemozoin retention and sequestration of parasitized red blood cells (Rupani and Amarapurkar, 2009; Whitten et al., 2011).

Even though liver malfunction has been reported to be marked as ‘malarial hepatitis’, histopathological findings of hepatocellular trauma have been modest. It has been noted that hepatocellular jaundice due to *P. falciparum* is not as a result of hepatitis rather may be due to the low clearance of bilirubin possibly as a consequence of the parasitaemia on the liver parenchymal cells, or metabolic acidosis, or endotoxemia or an amalgamation of these aberration (Anand and Puri, 2005). An examination of the liver tissue using electron microscopy in *falciparum* malaria revealed hyperplasia of kuppfer cells and macrophages found in the sinusoidal. Furthermore, modifications of the hepatocytes, mitochondria, endoplasmic reticulum sinusoidal experiencing diminished microvilli (De Brito et al., 1989) and bile canaliculi (Kochar et al., 2003) have been reported.

Although the occurrence of hepatocellular jaundice in complicated and severe malaria has not been shown in the histological analysis as fulminant liver failure, however, the current terminology of malarial hepatitis could be an inaccurate name rather malarial hepatopathy may be appropriate for the recent knowledge pertaining to hepatocellular malfunctions in malarial infected patients (Anand and Puri, 2005). In a separate article by Kochar et al. (2003), they presented evidence to the fact that malarial hepatitis should be perceived as a misnomer due to the fact that their study findings depicted increased levels of conjugated bilirubin as well as elevated enzymes such as ALT, AST, and LDH levels as well as necrosis in liver cells during histopathological analysis among patients with *Plasmodium falciparum* infection.
However, a particular assertion that cannot be challenged is the fact that malarial hepatitis mostly affects other organs substantially. According to Murthy et al., (1998), patients suffering from malaria hepatitis experienced substantial relative frequency of complexity, including respiratory distress, septicemia, renal dysfunction, and increased mortality cases. The above authors concluded that *falciparum* malaria depicts an increased incidence of complications with poor prognosis.

### 2.10.3 Management and Treatment

Even though the indication of hepatocellular jaundice denote the severity of malaria, the treatment of disease in that regard do not change but follows the standard treatment protocol; there is not yet any basis for the alteration of malaria treatment in such patients (Anand and Puri, 2005). In the sub-Saharan region, it is imperative for clinicians to have a high level of suspicion of the significant effect of malaria infection in the acute feverish state alongside with hepatic malfunction. During a differential diagnosis, another ailment prevalent in the sub-region such as enteric fever, leptospirosis and severe viral infection should be taken into cognizance (Bhalla et al., 2006). However, because clinical symptoms may not vary to a greater extent, laboratory assistance would be appropriate to ascertain the diagnosis of malarial hepatitis (Devarbhavi et al., 2005).

Sometimes, regular blood film examination for malaria parasites may not be adequate to diagnose an individual. According to White (1996), the analysis of malaria parasites for the first time was able to determine the diagnosis of 95% of individuals with severe malaria. In a related study by Bhalla et al. (2000), they concluded that three (3) sequential blood film examinations of malaria parasites produced 95% diagnosis of severe malaria among one hundred and fifty (150) subjects.
There is, therefore, the need for suitable tests to be done to rule out all other differential diagnoses. However, ultrasonography and Liver function tests are considered essential, but liver biopsy, not a necessity. On the other hand, protracted unascertained jaundice should prompt a clinician to initiate liver biopsy investigations (Wilairatana et al., 1994).

In endemic regions, the drug of preference for the management of *falciparum* malaria infection in the severe state is Intravenous artesunate thus for the first-line treatment while Artemether as well as Quinine are adopted as the second-line remedy (WHO, 2015). Furthermore, whenever the patient is able to orally take medications, especially after 24 hours of artesunate, ACT could be initiated for treatment completion (Plewes et al., 2018).

Mostly after treatment with the appropriate antimalarial drug, malarial hepatitis usually recedes after the elimination of the parasites within the body. The total serum bilirubin levels generally decrease at 72 hours of the commencement of treatment; nevertheless, it could be deferred among patients with co-morbid of renal malfunction (Kochar et al., 2003).
3.1 Study Design

A cross-sectional design to evaluate the effect of malaria infection on liver and kidney function among children under-five years.

3.2 Study Area

The study was conducted at Jasikan District Hospital, Oti - Region, from January to April, 2019. The Jasikan District Hospital has a fifty-three (53) bed capacity with malaria as its highest Out-Patient Department (OPD) case registered in 2017-2018 (Jasikan District Hospital Annual report, 2018). The district is situated in the northern part of the Volta region of Ghana. The District hospital serves about twenty (20) catchment areas including Jasikan being the largest and Lalakul the smallest in terms of population by sex and households. It shares boundary with Kadjebi District to the north, the Biakoye District to the west, the Hohoe Municipality in the south and to the east with the Republic of Togo. The district spread a total land area comprising of 555.8 square kilometers depicting 6.6%of land area for the Volta region (Ghana Statistical Service, 2012). The district profile is undulating and hilly in some areas. It is enveloped by the Buem-Togo mountain ranges. The eastern part of the district has comparatively higher altitudes with heights spanning from 260 to 680 meters which is higher in position of the sea level. The District dwells within the wet Equatorial Zone and has two main seasons, the wet and the dry, with the wet season spanning between May to September. The dry season ranges between December to April and manifests with cool and dry winds. According to the population and housing Census (2010), the district annual mean rainfall ranges between 1,250mm and 1,750mm. The district is predominantly made up of Buem tribe accounting for 65% of the inhabitants, the Ewe tribe accounting to 20% and Kotokoli chronicled as
The greater number (72.4%) of the district population lives in the rural communities as against the urban communities: Jasikan, Bodada, and Okadjakrom. Major occupations found in this district include agriculture, forestry and fishery as well as sales workers (Ghana Statistical Service, 2012).

Figure 3. 1 Map of Jasikan District. Source: Ghana Statistical Service (2012)

3.3 Study Population

The study population consisted of children between the ages of 6 to 59 months that were either malaria positive or negative. Out of the four hundred and nineteen (419) parents or guardians contacted in respect to the study, four hundred (400) consented for their wards to participate in the study.
3.4 Inclusion Criteria

Children aged 6 to 59 months with or without malaria infection were recruited after consent from parents/guardians.

3.5 Exclusion Criteria

Children between the ages of 6 to 59 months whose parent/guardian refused written consent were excluded from the study. Children with a pre-existing renal disease, hepatic disease, diabetes, human immunodeficiency virus and sickle cell diseases were also excluded from the study.

3.6 Controls

Participants who served as controls were screened to be sure they were without the presence of malaria parasites on both the thick and thin blood films.

3.7 Cases: criteria

In this study, the cases were defined as people with the presence of parasites on both thick and thin peripheral blood stained film.

3.8 Risk

There was no underlying risk related to this study on the participants. Unavoidable but slight discomfort such as pain and minimal bruising were observed during blood sampling by venipuncture. All standardized precautions were adhered to in mitigating such discomfort.

3.9 Ethical Clearance

Approval for the study was sought from the Ethical Review Committee of the University of Ghana College of Health Sciences (Protocol identification number: (CHS- Et/M.7-P5.11/2018-2019) (Appendix D). The study was explained in plain language to all
participants and their guardians. A written informed consent was obtained before initiating study procedures.

3.10 Sample Size Calculation

Malaria effects on liver and kidney among children less than five years was not known and hence a 50% prevalence rate was assumed in the calculation (Naing et al., 2006). The calculated sample size obtained was 384. A total of four hundred (400) participants were thus recruited for the study.

The minimum sample size was determined as described by Pourhoseingholi et al., (2013):

\[
n = \frac{z^2 p (1-p)}{d^2}
\]

- \(n\) = sample size,
- \(p\) = prevalence of u/5 malaria infection on renal and hepatic function
- \(d\) = margin of error (5%)
- \(Z\) = 1.96 at 95% confidence interval

3.11 Data Collection

Parents or guardian who gave their consent were given questionnaires. Data captured on the questionnaire included socio-demographics, knowledge, awareness and clinical signs of malaria in children.

3.12 Statistical Analysis

The data was summarized in Microsoft Excel (Office 2010) and analyzed using STATA v12. Socio-demographic and categorical variables were summarized with descriptive
statistics. Independent-sample t-tests were conducted to determine significant differences between two groups. MANOVA with post-hoc analysis were done where appropriate.

The results were expressed as mean ± standard deviation or in percentages. Statistical significance was set at $p < 0.05$.

### 3.13 Collection of Blood

A measured volume of four milliliters (4mls) of blood were collected by venipuncture from each child by a phlebotomist using five (5) ml needle and syringe. One (1) ml of whole blood was immediately transferred into ethylene diamine tetra acetic acid (EDTA) containing tube for parasitological studies, while the remaining 3mls were transferred into a plain tube, allowed to clot and processed. The serum was separated from the clotted blood using a centrifuge at 3000rpm for 5 minutes. The separated serum was used for analysis the same day. The analytes were determined using ELITech reagents and controls with the use of an automated Analyzer (Junior Selectra Chemistry analyzer). Total protein, albumin, creatinine, urea, liver transaminases (ALT, AST), alkaline phosphatase and bilirubin (total and direct) were determined from sera.

### 3.14 Parasitological Analysis

#### 3.14.1 Staining Thick and Thin blood films for Microscopy

For the determination of parasitaemia, thick and thin blood films were prepared for all the participants on a new, clean, grease-free, unscratched and well labeled slide based on the guidance of Agomo et al., (2001). In the preparation of the thick blood film, approximately 6µl of blood was placed on the clean grease-free slide using a micropipette. With the use of a beveled corner of the spreader slide, blood was spread evenly in a circular pattern to cover a diameter of 1-2cm on the slide. Precautions were
taken to ensure that the film would not be overly thick such that a news print cannot be read through or hands of a watch cannot be read through. In the preparation of the thin blood film, approximately 2-3µl of blood was placed on the slide using a micropipette. With the use of a ground edge of a spreader slide, the blood was spread steadily along the slide at an angle of 45 degrees. The peripheral blood films (thick and thin) prepared were air dried. The thin film was fixed by the use of an absolute methanol for about 2 seconds and then allowed to air dry. The films were then ready for staining. The blood films were then stained with 10% Giemsa stain for 10 minutes. After the ten (10) minutes it was washed in buffered water at a pH of 7.2. The back of the stained glass slide was cleaned with gauze and the slide was placed upright on the drying rack. Blood films were allowed to air dry at room temperature devoid of dust and direct sunlight for at about 20 minutes. After, the peripheral blood films were observed under the microscope for the detection of malaria parasites in conformity to WHO standards (WHO, 2010). A drop of immersion oil was placed on the stained slide and was placed under the microscope, and examined with an objective lens. The field of the slide was examined in a battlement manner. With the use of two tally counters (one for parasites count and the other for WBC count), the trophozoites or the ring forms of the parasite were determined separately as well as the white blood cells. Counting of the parasites and the WBC continues until the counting of WBC’S reaches two hundred (200). For all malaria positive cases, malaria parasites density was ascertained using the thick film. The parasite count was expressed in microliter as standard white cell count (8000/µl) or the actual white cell count of the patient by the parasite count divided by the 200 WBC counted.

Parasite density count was expressed as:

Parasite density per µL = Total number of parasites counted × WBC count per µL ÷ Number of WBCs counted.
3.15 Biochemical Analysis

3.15.1 Kidney Function test

Kidney function test was done by determining the serum creatinine and urea levels among all participants. The procedure and the principles are outlined below.

3.15.2 Determination of Creatinine

3.15.3 Method:

The serum creatinine was determined by the colorimetric, Jaffe-kinetic method using an automated analyzer, Junior Selectra and Elitech reagents from France.

3.15.4 Principle:

An alkaline picrate reacts with creatinine resulting in the formation of a coloured red complex. The use of the kinetic procedure reduces the effect of interfering substances. The colour intensity formed is correlative to the concentration of creatinine in the sample.

3.15.5 Reaction:

\[
\text{Creatinine + picric acid} \xrightarrow{\text{Alkaline pH}} \text{Colored red complex}
\]

3.15.6 Procedure:

A measured volume of 0.5ml of serum was dispensed into a labeled pediatric cup and loaded onto the sample rotor on the automated analyzer (Junior Selectra). The patient’s information was imputed into the analyzer and the test was initiated to run. Working reagent such as reagent 1(picric acid) and reagent 2 (sodium hydroxide and Disodium phosphate) were mixed together in equal volume (125µl) with the sample (25µl) by the analyzer. The concentration was read at 505nm. Automatic results were generated on the screen of the analyzer and the results were printed out.
3.16 Determination of Urea

3.16.1 Method:
Serum urea was measured by the enzymatic UV-Kinetic method using the Junior Selectra automated analyzer for analysis.

3.16.2 Principle:
In the presence of urease, urea is hydrolysed to ammonia and carbon dioxide. In the presence of reduced nicotinamide-adenine dinucleotide and glutamate dehydrogenase, ammonium ions react with \( \alpha \)-ketoglutarate to form L-glutamate and nicotinamide adenine dinucleotide (NAD\(^+\)).

3.16.3 Reaction:

\[
\text{Urea} + 2\text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_4^+ + \text{CO}_3^{2-}
\]

\[
\text{NH}_4^+ + \alpha\text{-ketoglutarate} + \text{NADH} \xrightarrow{\text{GIDH}} \text{L-Glutamate} + \text{NAD}^+ + \text{H}_2\text{O}
\]

GIDH= Glutamate dehydrogenase.

3.16.4 Procedure:
A volume of 0.5 ml was dispensed into a well labeled pediatric cup with a pipette. The cup was loaded onto the sample rotor in the analyzer. Participant’s information was imputed and the test to be analysed was selected. An inbuilt pipette, pipetted 240µl of the Reagent 1 (Urease, \( \alpha \)-ketoglutarate, GIDH, sodium azide, Tris buffer, and ADP) loaded on board of the analyzer was mixed internally in a cuvette with 3 µl of the sample for 4 minutes. A minimum volume of 60µl of Reagent 2 (NADH, sodium azide) was mixed with the reagent 1 with the sample at a wavelength of 340nm. The test was read against a reagent blank. Results were generated by the analyzer in a print out.
3.17 Liver Function test

Liver function test was done by determining in serum ALT, AST, ALP, Bilirubin, and Total protein concentrations of subjects. The procedure and the principles are found below.

3.17.1 Determination of Alanine aminotransferase (ALT)

3.17.2 Method:

The serum ALT was measured on the Junior Selectra analyzer using the IFCC kinetic method without pyridoxal phosphate (P-5-P).

3.17.3 Principle:

During the kinetic determination of alanine aminotransferase (ALT) activity, in the presence of ALT, L- Alanine reacts with α-ketoglutarate to form pyruvate and L-Glutamate. In the second reaction, pyruvate reacts with reduced nicotinamide dinucleotide (NADH) in the presence of lactate dehydrogenase (LDH) to form L-lactate and NAD.

3.17.4 Reaction:

\[
\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}
\]

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^+
\]

3.17.5 Procedure:

A volume of 0.5 ml of serum was dispensed into a labeled paediatric cup. The pediatric cup was loaded on board of the analyzer. All the necessary bio data of the subjects were imputed into the analyzer. The test analysis was initiated to run. The analyzer with an inbuilt pipette mixed the on board reagent I (Tris buffer, LDH, L-Alanine, and Sodium azide) 240µl and reagent 2 (α-ketoglutarate, NADH, Sodium azide) 60 µl as well as the
3.18 Determination of AST (Aspartate aminotransferase)

3.18.1 Method: The serum AST was measured on the Junior Selectra analyzer using the International Federation of clinical chemist (IFCC) kinetic method without pyridoxal phosphate (P-5-P).

3.18.2 Principle

During the kinetic determination of aspartate aminotransferase activity, the amino group of L-aspartate is catalyzed by AST to α-ketoglutarate to form L-glutamate. In the second reaction, oxaloacetate and reduced NAD reacts in the presence of malate dehydrogenase (MDH) to form malate and NAD.

3.18.3 Reaction:

\[
\text{AST} \quad \text{L-aspartate} + \alpha\text{-ketoglutarate} \quad \text{Oxaloacetate + L-Glutamate}
\]

\[
\text{MDH} \quad \text{Oxaloacetate + NADH + H+} \quad \text{L-Malate + NAD}^+
\]

3.18.4 Procedure:

A measured volume of 0.5 ml of serum was dispensed into a labeled paediatric cup. The pediatric cup was loaded on board of the analyzer. All the needed bio data of the participants were imputed into the analyzer. The test analysis was initiated to run. The analyzer with an inbuilt pipette mixed the on board reagent I (L-Aspartate, Tris buffer, LDH, MDH, Sodium azide) 240µl and reagent 2 (α-ketoglutarate, NADH, Sodium azide) 60µl as well as the sample 30µl to measure the absorbance. The results were generated in a print out from the analyzer.
3.19 Determination of Alanine phosphatase (ALP)

3.19.1 Method:
The serum ALP was determined based on the German Society of Clinical Chemistry (DGKC) and the Scandinavian Society of Clinical Chemistry (SCE) enzymatic. Kinetic method.

3.19.2 Principle:
In the presence of diethanolamine and Mg$^{2+}$ as acceptors of phosphate, there is the transformation of p-nitrophenylphosphate by alkaline phosphatases to phosphate and p-nitrophenol a yellowish compound.

3.19.3 Reaction:

\[
\text{Alkaline Phosphatase} \quad \text{p- Nitrophenylphosphate + H}_2\text{O} \rightarrow \text{Inorganic phosphate + p- Nitrophenol}
\]

3.19.4 Procedure:
A measured volume of 0.5 ml of participant’s serum was dispensed into a labeled pediatric cup. The pediatric cup was loaded on board of the analyzer. Participant’s information was programmed into the analyzer. The test analysis was initiated to run. The analyzer with an inbuilt pipette mixed the on board reagent I (Diethanolamine, Magnesium Chloride, and Sodium azide) 200µl and reagent 2 (p-Nitrophenylphosphate and sodium azide) 50µl as well as the sample 5µl to measure the absorbance. The results were generated in a print out from the analyzer after 133 seconds.

3.20 Determination of Bilirubin (total and direct)

3.20.1 Method: The method used in the analysis of Bilirubin (total and direct) was Malloy Evelyn Modified. Endpoint.
3.20.2 Principle:
Sulphanilic acid reacts with sodium nitrite to form diazotized sulphanilic. Conjugated and unconjugated bilirubin reacts with diazotized sulphanilic acid to form azobilirubin in the presence of centrimide (an accelerator). Without the accelerator, only the conjugated bilirubin would react. An increase in the absorbance at 546nm is proportional to bilirubin concentration.

3.20.3 Reaction:

\[
\text{Sulphanilic acid} + \text{NaNO}_2 \rightarrow \text{Diazotized sulphanilic acid}
\]

\[
\text{Bilirubin} + \text{Diazotized sulphanilic acid} \rightarrow \text{Azobilirubin}
\]

3.20.4 Procedure:

A measured volume of 1 ml of participant’s serum from the plain tube was dispensed into a labeled pediatric cup. The pediatric cup was loaded on board onto the analyzer. Participant’s information was programmed into the analyzer. The test analysis was initiated to run. The analyzer with an inbuilt pipette mixed the on board reagent 1 (sulphanilic acid and cetrimide) 240µl of the total bilirubin with the 15µl of the sample and 60µl of reagent 2 (total and direct bilirubin, sodium nitrite) to measure the absorbance at a wavelength of 546-700nm. The results were generated in a print out from the analyzer after 6 minutes.

With direct bilirubin, 240µl of reagent 1(sulphanilic acid) was dispensed into an inbuilt cuvette using an inbuilt pipette. The dispensed reagent 1 was mixed with the 30µl of the sample within the analyzer. After four minutes of incubation in the analyzer, 60µl of reagent 2 was added and the absorbance was read at 546nm after 50 seconds.

Indirect bilirubin was calculated from the total and direct bilirubin by the analyzer.
3.21 Determination of Total Protein

3.21.1 Method:
In the determination of serum total protein, Biuret. Endpoint method was used in the analysis.

3.21.2 Principle:
In the presence of copper salt in an alkaline solution, serum proteins form a coloured complex.

3.21.3 Reaction:

\[
\text{Alkaline solution} \\
\text{Proteins} + \text{Cu}^{2+} \rightarrow \text{Coloured complex}
\]

3.21.4 Procedure:
A measured volume of 0.5ml of the serum was dispensed into a pediatric cup. The pediatric cup was loaded onto the analyzer sample rotor. Reagent R(Potassium iodide, Potassium sodium tartrate, copper sulfate and sodium hydroxide) already loaded on board of the rotor was mixed together with 3µl of the sample with an aid of an inbuilt pipette. The absorbance was read after 11 minutes and the results were recorded in the analyzer alongside a printed out results.

3.22 Determination of Albumin
3.22.1 Method:
In the determination of serum albumin Colorimetric- Bromocresol green (BCG) was used in the analysis.

3.22.2 Principle:
Bromocresol green at pH4.20 was used in the colorimetric determination of albumin.

3.22.3 Reaction:

\[
\text{pH} = 4.20 \\
\text{Albumin} + \text{BCG} \rightarrow \text{Albumin-BCG complex}
\]
3.22.4 Procedure:
A measured volume of 0.5ml of the serum was dispensed into a pediatric cup. The pediatric cup was loaded onto the analyzer sample rotor. The analyzer with an inbuilt pipette mixed the on board reagent R (succinate buffer, Bromocresol green, and Brij 35) 360µl of the albumin reagent with the 3µl of the test. The absorbance was read at 620 nm at a temperature of 37°C after 4 minutes of incubation. The results were recorded in the analyzer alongside a printout results.

3.23 Quality Control
To enhance the accuracy of assays, control sera such as Elitrol 1(normal control) and Elitrol 2 (abnormal control) and calibrators such as Elical 2 were used. The use of these controls were performed and validated prior to the assaying of patients sample. Hence the control frequency was done anytime participants samples were about to be assayed. Results obtained for the control were within range; hence no corrective measures were taken.
4.0 RESULTS

4.1 Demographic characteristics of the participants

Table 4.1 depicts demographic characteristics of the participants. A total of four hundred (400) participants comprising two hundred cases and two hundred controls were involved in this study. The case groups were further grouped as mild (160) and severe (40). The mean age (months) of the participants were 24.5 ±14.2. For gender, there were more males (54.5%) compared to females (45.5%).

Table 4.1 Demographic characteristics of participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n=400)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean (sd) months</td>
<td>24.5 (14.2)</td>
<td></td>
</tr>
<tr>
<td>Childs Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>198</td>
<td>49.5</td>
</tr>
<tr>
<td>&gt;2</td>
<td>202</td>
<td>50.5</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>182</td>
<td>45.5</td>
</tr>
<tr>
<td>Male</td>
<td>218</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Table 4.1 shows the summary characteristics of participants. SD of age in parenthesis.

Table 4.2 Demographic characteristics of parents/guardians

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n=400)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guardians' Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>84</td>
<td>21</td>
</tr>
<tr>
<td>25-34</td>
<td>222</td>
<td>55.5</td>
</tr>
<tr>
<td>&gt;35</td>
<td>94</td>
<td>23.5</td>
</tr>
<tr>
<td>Level of Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal</td>
<td>106</td>
<td>26.5</td>
</tr>
<tr>
<td>Basic</td>
<td>86</td>
<td>21.5</td>
</tr>
<tr>
<td>Secondary</td>
<td>150</td>
<td>37.5</td>
</tr>
<tr>
<td>Tertiary</td>
<td>58</td>
<td>14.5</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>58</td>
<td>14.5</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Married</td>
<td>314</td>
<td>78.5</td>
</tr>
<tr>
<td>Religion</td>
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</tr>
<tr>
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<td>302</td>
<td>75.5</td>
</tr>
<tr>
<td>Muslim</td>
<td>90</td>
<td>22.5</td>
</tr>
<tr>
<td>Traditional</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4.2 shows the summary characteristics of parents and guardians.
Majority of the guardians were within the age bracket of 25-34 years representing 55.5%.

An appreciable number of the guardians (37.5%) had secondary education at the time of participating in the study; whiles about 26.5% had no formal education. About 2/3rd (78.5%) of the guardians were married. In terms of religion, Christianity formed the majority (75.5%) among the parents or guardians of the participants (Table 4.2).

### 4.2 Knowledge and identification of malaria infection among Participants

Blood films were examined among all the four hundred (400) participants. Generally, 200 (50%) participants were negative for malaria and were used as controls. Two hundred of the participants were confirmed positive for malaria infection and they served as the case group. Case subjects were further classified as mild infection 160 (40%) with parasitaemia levels less than 100,000 parasites per µl of blood whilst 40 (10%) of the subjects were considered as severe infection with parasitaemia levels above 100,000 parasites per µl of blood (Figure 4.1 and Table 4.3). Among the participants, 230 (57.5%) experienced fever episodes, whilst 170 (42.5) were without fever episodes (Table 4.2). A total of 278 (69.5%) of the respondents had knowledge about the causative agent of malaria, whiles the rest (30.5%) had no knowledge about the causative agent of malaria.

Of the four hundred (400) participants, 84% had ownership of insecticide treated net (ITN). About 81.5% of the number that owns the ITN confirmed its usage in their homes, whilst 18.5%, although owed the ITN, did not use it (Table 4.3).
Table 4.3 Characteristics of malaria infection among participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n=400)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fever episodes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>170</td>
<td>42.5</td>
</tr>
<tr>
<td>Yes</td>
<td>230</td>
<td>57.5</td>
</tr>
<tr>
<td><strong>Causes of malaria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coughing</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Dirty Environment</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>High temp</td>
<td>16</td>
<td>4.0</td>
</tr>
<tr>
<td>Mosquito</td>
<td>278</td>
<td>69.5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8</td>
<td>2.0</td>
</tr>
<tr>
<td>No idea</td>
<td>86</td>
<td>21.5</td>
</tr>
<tr>
<td><strong>ITN ownership</strong></td>
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<td></td>
</tr>
<tr>
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<td>64</td>
<td>16.0</td>
</tr>
<tr>
<td>Yes</td>
<td>336</td>
<td>84.0</td>
</tr>
<tr>
<td><strong>ITN usage (n=336)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=336)</td>
<td>62</td>
<td>18.5</td>
</tr>
<tr>
<td>Yes</td>
<td>274</td>
<td>81.5</td>
</tr>
<tr>
<td><strong>Severity of Malaria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>50.0</td>
</tr>
<tr>
<td>Mild</td>
<td>160</td>
<td>40.0</td>
</tr>
<tr>
<td>Severe</td>
<td>40</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 4.2 shows summary characteristics of knowledge and identification of malaria among participants and their guardians. (ITN- Insecticide Treated Net).

![Figure 4.1 Parasitaemia count by severity of infection](image_url)

Figure 4.1 Parasitaemia count by severity of infection
4.3 Kidney function test, total protein, albumin and malaria infection

The mean urea levels for the mild (5.0 ± 1.7) and severe (11.6 ± 4.9) groups were respectively higher \((p<0.05)\) when compared with the control group (2.8 ± 0.8) (Table 4.4). Creatinine concentrations for mild and severe groups were higher \((p<0.05)\) respectively when compared with their control counterparts. Further, within the case group, creatinine levels were significantly higher \((p<0.05)\) in the severe group relative to the mild group (Table 4.4). A post-hoc analysis revealed significant difference in serum urea and creatinine levels among the mild versus severe groups \((p<0.001)\) and severe versus control groups \((p<0.001)\) (Table 4.6). Using the Pillais trace \((V)\), there was a significant effect of malaria infection on both serum urea and creatinine \((p<0.01)\) (Table 4.5). The serum total protein and albumin values were relatively lower in the control group compared with mild and severe groups \((p<0.05)\) (Table 4.6).

Table 4.4 Effects of malaria infection on renal function test, total protein and albumin

<table>
<thead>
<tr>
<th>Malaria Infection</th>
<th>No: (400)</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (µmol/l)</th>
<th>Total protein (g/l)</th>
<th>Albumin (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>200</td>
<td>2.8 ± 0.8</td>
<td>25.3 ± 5.3</td>
<td>78.7 ± 4.2</td>
<td>47.2 ± 3.6</td>
</tr>
<tr>
<td>Mild</td>
<td>160</td>
<td>5.0 ± 1.7(^\text{A})</td>
<td>52.3 ± 18.2(^\text{A})</td>
<td>72.7 ± 6.2(^\text{A})</td>
<td>44.0 ± 5.1(^\text{A})</td>
</tr>
<tr>
<td>Severe</td>
<td>40</td>
<td>11.6 ± 4.9(^\text{A})</td>
<td>125.7 ± 60.1(^\text{A})</td>
<td>60.8 ± 8.0(^\text{A})</td>
<td>34.8 ± 9.0(^\text{A})</td>
</tr>
</tbody>
</table>

Values are represented as mean ± standard deviation. \((A)\) denotes significance when compared to control. \(p< 0.05\) was considered significant.
Table 4.5 Multivariate Analysis of Variance (MANOVA) of the effects of malaria infection on kidney function test

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df</th>
<th>F(df1, df2)</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>0.2656</td>
<td>2</td>
<td>6.0 390.0</td>
<td>61.13 &lt;.001</td>
</tr>
<tr>
<td>P</td>
<td>0.7447</td>
<td>6.0 392.0</td>
<td>38.76 &lt;.001</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>2.7268</td>
<td>6.0 388.0</td>
<td>88.17 &lt;.001</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>2.7126</td>
<td>3.0 196.0</td>
<td>177.22 &lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5 shows a multivariate analysis of variance of the effects of malaria on urea and creatinine. W = Wilks' lambda, P = Pillai's trace, L = Lawley-Hotelling trace, R = Roy's largest root. (These four (W, P, L, R) statistic test were used to determine significance based on the F-distribution since multivariate statistic is not direct hence it is dependent on the above four statistics used).

Table 4.6 Adjusted multiple comparisons of the effects of malaria infection on kidney function test, total proteins and albumin

<table>
<thead>
<tr>
<th>Malaria infection</th>
<th>Difference</th>
<th>95% CI</th>
<th>Duncan</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>2.2</td>
<td>1.63 2.79</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>8.8</td>
<td>7.82 9.79</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>6.5</td>
<td>5.63 7.55</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>27.0</td>
<td>20.44 33.61</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>100.4</td>
<td>89.09 111.74</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>73.3</td>
<td>62.41 84.372</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>-6.0</td>
<td>-7.60 -4.40</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>-17.9</td>
<td>-20.65 -15.14</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>-11.8</td>
<td>-14.56 -9.22</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>-3.2</td>
<td>-4.7 -1.7</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>-12.5</td>
<td>-14.9 -10.0</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>-9.2</td>
<td>-11.9 -6.8</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6 depicts multiple comparisons or the post hoc analysis of the effects of malaria on urea, creatinine, total protein and albumin. Significant differences (P < 0.05) were observed among the cases and the control comparisons.
4.4 Effects of malaria infection on Liver Function Test

From table 4.7, the serum AST and ALT levels were relatively lower among the control groups (Mean= 24.8, SD= 6.7) than the mild (Mean=47.2, SD= 14.4) and severe groups (Mean=103, SD= 37.4) respectively. Serum ALP levels were relatively high in the mild group (Mean =561.7, SD= 196.5) than in the control (Mean= 463.8, 151.2) and severe groups (Mean=476.5, SD =82.2). Serum total bilirubin was higher in the severe group (Mean=114.2, SD= 92.1) relative to the control (Mean=10.9, SD= 2) and mild groups (SD= 27.6, SD= 14.1) respectively (Table 4.7)

Using the Pillai’s trace (Table 4.8) there was a significant difference of malaria infection on AST, ALT, ALP, and Bilirubin (V= 0.95, F (10, 388) =35.6, P < 0.001) (table 4.8). Post-hoc comparisons revealed significant differences in serum AST, and ALT among the mild versus control group (p<0.001), severe versus control group (p<0.001), as well as severe versus mild groups (p<0.001) respectively. With respect to serum ALP, post-hoc analysis revealed a significant difference between the mild versus control (p<0.001), but not between the severe versus control groups (p<0.756). The serum direct bilirubin was different for mild versus control (p<0.001), severe versus control (p<0.001) and severe versus mild (p<0.001) (Table 4.9).
Table 4.7 Summary statistics of liver function test by severity of malaria infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Liver function test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST (U/l)</td>
<td>ALT (U/l)</td>
<td>ALP (µmol/l)</td>
<td>B.Tot. (µmol/l)</td>
<td>B. Indir. (µmol/l)</td>
<td>B. Dir. (µmol/l)</td>
</tr>
<tr>
<td>Malaria infection</td>
<td>Control</td>
<td>Mean</td>
<td>24.8</td>
<td>20.7</td>
<td>463.8</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>6.7</td>
<td>6.3</td>
<td>151.2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>Mean</td>
<td>47.2</td>
<td>37.9</td>
<td>561.7</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>14.4</td>
<td>14</td>
<td>196.5</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Mean</td>
<td>103</td>
<td>78.2</td>
<td>476.5</td>
<td>114.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>37.4</td>
<td>27.9</td>
<td>82.2</td>
<td>92.1</td>
</tr>
</tbody>
</table>

Table 4.7 depicts effects of malaria infection on serum AST, ALT, ALP, and Bilirubin (total and direct). B.Tot (Bilirubin total), B.Indir. (Bilirubin indirect), B.Dir. (Bilirubin direct)

Table 4.8 Multivariate Analysis of Variance (MANOVA) of the effects of malaria infection on Liver function

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df</th>
<th>F(df1, df2)</th>
<th>F -test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>0.1814</td>
<td>10.0 , 386.0</td>
<td>52.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>P</td>
<td>0.9575</td>
<td>10.0 , 388.0</td>
<td>35.64</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 4.8 shows a multivariate analysis of variance of the effects of malaria on AST, ALP, ALT, and Bilirubin levels. W = Wilks' lambda, P = Pillai's.
Table 4.9 Adjusted multiple comparisons of the effects of malaria infection on liver function test

<table>
<thead>
<tr>
<th>Malaria infection</th>
<th>Difference</th>
<th>t</th>
<th>Lower</th>
<th>Upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AST (U/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>22.4</td>
<td>9.6</td>
<td>17.85</td>
<td>27.04</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>78.1</td>
<td>20.6</td>
<td>70.29</td>
<td>86.08</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>55.7</td>
<td>14.4</td>
<td>48.08</td>
<td>63.39</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>AST (U/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>17.2</td>
<td>8.7</td>
<td>13.32</td>
<td>21.12</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>57.4</td>
<td>17.8</td>
<td>50.77</td>
<td>64.15</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>40.2</td>
<td>12.2</td>
<td>33.76</td>
<td>46.74</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>ALP (U/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>97.8</td>
<td>3.9</td>
<td>46.07</td>
<td>149.58</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>12.6</td>
<td>0.3</td>
<td>-67.63</td>
<td>92.94</td>
<td>0.756</td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>-85.1</td>
<td>-2.1</td>
<td>-167.11</td>
<td>-3.23</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>BIL TOT (µmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>16.7</td>
<td>3.7</td>
<td>7.89</td>
<td>25.63</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>103.3</td>
<td>14.1</td>
<td>88.13</td>
<td>118.62</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>86.6</td>
<td>11.6</td>
<td>71.82</td>
<td>101.39</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>BIL. IND (µmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>4.7</td>
<td>1.2</td>
<td>-3.43</td>
<td>12.95</td>
<td>0.253</td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>37.9</td>
<td>5.6</td>
<td>23.86</td>
<td>52.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>33.1</td>
<td>4.8</td>
<td>19.54</td>
<td>46.82</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>BIL. DIR(µmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild vs Control</td>
<td>12.0</td>
<td>6.1</td>
<td>8.12</td>
<td>15.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Control</td>
<td>65.4</td>
<td>20.3</td>
<td>58.76</td>
<td>72.12</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Severe vs Mild</td>
<td>53.4</td>
<td>16.3</td>
<td>46.96</td>
<td>59.92</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 4.9 depicts multiple comparisons or the post hoc analysis of the effects of malaria on liver enzymes and bilirubin concentrations. \( P<0.05 \) was considered significant.
CHAPTER FIVE

5.0 DISCUSSION

5.1 Impact of Malaria on urea, creatinine, total proteins and albumin

Malaria infection among children contributes to high infection rate in the world, principally in the endemic regions such as the sub-Saharan (WHO, 2010). Most of the complexity and death that manifest due to the disease among children are as a result of \textit{P. falciparum}. Amidst the complications connected to \textit{P. falciparum} include renal and hepatic dysfunctions predominantly in adults and some occurrence in children living in endemic malaria regions (Nonvignon \textit{et al}., 2016; Afoakwa \textit{et al}., 2018). Impairment in these two significant organs could be extremely devastating; hence, prompt detection and management of the ailment should be of utmost importance.

This study demonstrated a significant increase in serum urea and creatinine levels among children with severe malaria infection relative to the mild and control groups (Table 4.4; 4.6). Higher levels of urea and creatinine among the case group may imply that severity of malaria infection has consequences on the levels of serum urea and creatinine among infected persons. The movement of \textit{P.falciparum} merozoites into the erythrocytes relies on ligand- receptor interplay between the pointed end of the parasite and the red blood cells. It is this interaction that the parasite relies on to penetrate into the RBC to be matured. This phenomenon leads to the alteration and deformability of the erythrocytes membrane (Cowman \textit{et al}., 2016). This further leads to cytoadherence of the erythrocytes to the vascular endothelium and subsequently results in impairment of the microcirculation, by forming rosettes or clumps; the concomitant effects of a reduced renal perfusion as a result of the obstruction in the microcirculation leads to the activation of renin-angiotensin-aldosterone system and the subsequent release of renin by the justaglomerulus apparatus as well as the release of aldosterone from the zona
glomerulosa of the adrenal cortex. These actions together with the counter current multipliers, anti-diuretic hormone and urea recycling leads to oliguria and hence concentrates urea and creatinine in serum. Taken together, the above phenomena could lead to acute kidney injury and end-organ malfunction (Plewes et al., 2018). Again the apparent elevation of serum creatinine and urea especially among the severe group could also be due to the blockade of the microcirculation in the kidney as a result of the parasite erythrocytes sequestration leading to ischemia and immune-mediated glomerular injury being the likely mechanisms (Koopmans et al., 2015; Zaki et al., 2013). In a study by Plewes et al., (2018), he reported of an increase in serum creatinine in an acute malaria infection which rebound to normal levels after recuperation. This could explain what was observed in our cohort. A prior study has also reported of high serum creatinine and urea in severe malaria-infected children (Akanbi et al., 2015). Gomes et al., (2011) also indicated an acute renal failure in malaria with the presence of oliguria and elevated levels of serum urea and creatinine among the infected individuals. This study is in harmony with the above research observations indicating high serum urea and creatinine among the infected malaria cases relative to the control.

The significantly reduced serum total protein and albumin levels observed among the severe group relative to the mild and control groups could be as a result of glomerulonephritis which is prevalent in children with acute kidney injury a consequence of \textit{P. falciparum} infection (Koopmans et al., 2015; Silva et al., 2017). Another mechanism that could have resulted in the reduction of serum proteins among the case group include the release of cytokines (IL -1, IL6 and TNF) by macrophages during malaria infection, leading to decrease hepatic synthesis of albumin (50-60% of total proteins) hence reducing serum concentrations of total proteins and albumin (Graninger et al., 1992). This study agrees with previous studies that reported of low levels of serum
proteins and albumin in malaria-infected individuals (Akanbi et al., 2014; Akanbi et al., 2015). I was however unable to measure these inflammatory markers in this present study.

5.2 The effects of malaria on liver function test

The observed significant elevation of serum bilirubin levels (Table 4.9) among the children infected with the disease in this study may demonstrate increased hemolysis of the red blood cells as a result of the breakdown of parasitized and non-parasitized erythrocytes as well as hepatocyte injury. Typically, elevated serum bilirubin levels in malaria have been associated with biliary tract obstruction, hepatocellular injury, neonatal jaundice, haemolysis and cholestasis (Renner, 1995; WHO, 2000). The bile stasis may be due to impairment of the transport of bilirubin as a result of the obstruction of the endothelium and the derangement of the hepatocyte microvilli (WHO, 2000). The hepatocyte dysfunction may result from the changes in the vascular flow through the parenchymal cells while parasitised erythrocytes attach to the endothelial cells hence obstructing the sinusoids and inhibiting the intrahepatic flow of blood (Kockar, 2003). A finding by Yokoto and Calisei (2006) revealed an increased serum bilirubin among malaria-infected patients and ascribed the reasons to hemolysis occurring in the blood vessels with associated kidney failure. Many studies adduce hepatocellular injury among patients with elevated bilirubin of greater extent. Glor (1969) and Bartelloni (1967) indicated that mild jaundice among malaria patients might not be principally hemolysis alone, but rather a high degree of elevated serum bilirubin levels typifies hepatocyte malfunction. In this study, the observed elevation of bilirubin was of both conjugated and unconjugated origin. This then indicates the role of both hemolysis and hepatocytic damage as a cause. The above observations of the study bolster the involvement of hepatic dysfunction as the cause of elevated bilirubin levels among the subjects.
The observed elevation of liver transaminases (ALT and AST) among the severe and the mild group (cases) and the elevation of ALP among the malaria parasite-infected patients relative to the control group (Table 4.9) in this study depicts that, malaria parasite infection could be a contributory factor for the elevation of the liver transaminases and ALP, and could lead to hepatocellular injury. The observed increase of serum AST and ALT among the severe and mild groups may be due to the consequence of the damaged hepatocytes during the liver stage of the malaria parasites lifecycle and sinusoidal congestion, hence leading to the release of these enzymes into general circulation. Again, the elevated levels of AST and ALT in the severe group relative to the mild group could depict the indication that hepatocyte injury may be dependent on the level of parasitaemia in the blood. The study agrees with previous studies that revealed a positive interconnection between the level of parasitaemia and liver malfunction (Onyesom & Onyemakonor, 2011). Liver malfunctions are comparatively common manifestations of *P. falciparum* malaria, and it has been evidenced that atypical liver function profiles persist among infected patients (Wilaratna *et al.*, 1994). This observation in this study is in concurrence with previous studies indicating the role of *P. falciparum* infection in the elevation of liver enzymes (Onyesom & Onyemakonor, 2011).
CHAPTER SIX

6.0 CONCLUSION, LIMITATION AND RECOMMENDATION

6.1 CONCLUSION

This study observed that malaria infection is associated with alterations in the function of the liver and kidney of children less than five years, specifically among the infected individuals. The combination of elevated levels of urea, creatinine, liver transaminases (AST and ALT), bilirubin, reduced total protein and albumin, were significant predictors of adverse outcome of malaria infection among children under 5 years.

6.3 LIMITATION

There were three limitations noted in this study. Firstly, the inability to follow-up on the subjects to ascertain the extent of dysfunction the ailment had on the kidney and liver. This is partly due to the nature of the study design used. The next limitation was the period when recruitment was done. There was low incidence of the disease since it was in the low peak season hence contributing to the minimal number of the severe groups in the study. Furthermore, the inability to measure electrolytes, acute phase proteins as well as renal injury biomarkers (KIM-1 and NGAL) prevented us from drawing definitive conclusions.

6.2 RECOMMENDATION

There is the need to employ adequate intervention and control measures to manage the incidence of falciparum malaria and to minimize renal and kidney dysfunction. Prompt institutional (hospital) standards, including rapid malaria testing should be conducted for every child less than five years and adequate treatment initiated where necessary to reduce the impact of malaria infection on multiple organs. During antenatal visits and postnatal visits, the use of insecticide-treated net must be highlighted to encourage routine usage among mothers and children.


PARTICIPANTS INFORMATION SHEET

STUDY TITLE: Evaluation of Renal and Hepatic Dysfunction Among Children Less than Five Years With Malaria in Jasikan District, Oti Region - Ghana.

LOCALITY: Jasikan District

LEAD INVESTIGATOR: Isaac Djan Asare

Your authorization is being sought to have your child partake in this study. Kindly read carefully the following information before deciding whether to give your approval or otherwise.

Purpose of Research: The purpose of this study is to help us determine whether malaria infection has an effect on the liver and kidney of children in Jasikan District.

Procedure to be followed: During the procedure, blood will be collected via venipuncture from your child by trained Scientist/Technologist/Technician. The blood sample will be used for analysis to determine the presence of malaria and its effects on the liver and kidney.

Discomfort/Risks: 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). It might get a little bit hard and red at the site where the needle goes in. That should go away in a day. If it hurts longer than that, swells up, please endeavour to let us know. There are no foreseeable
dangers to either you or your child in this study during the processing of your child’s blood sample.

**Incentives/benefits for participation:** There are no direct benefits to you or your child. However, this study will help to enhance knowledge on how to care for children that are infected with malaria with kidney and liver dysfunctions.

**Time/duration of participation:** Participation in this study will not exceed more than 20 minutes.

**Statement of Confidentiality:** All data/records are kept highly confidential and will only be made available to professional researchers. If the results of this study are published, the data generated will be presented in group form and individual children will not be identified.

**Voluntary Participation:** Your child’s participation is voluntary. If you feel your child has in any way been coerced into participation, please inform the Lead researcher. We also ask that you read this letter to your child (if age-appropriate) and inform your child that participation is voluntary. At the time of the study, your child will once again be reminded of this by the researcher.

**Termination of participation:** You or your child is permitted at any point of the study to terminate the session and we will oblige accordingly.

**Questions relating to this research should be directed to:**

Isaac Djan Asare (0544862161)

Lead Researcher

adjan56@yahoo.co.uk
Please tick to indicate your consent to the following.

| 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 in this study. | Yes □ No □ |
| I am satisfied with the answers I have been given regarding the study. | Yes □ No □ |
| I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care. | Yes □ No □ |
| I consent to the research staff collecting and processing my child’s information, including information about his/her health. | Yes □ No □ |

Declaration by Parent/Guardian:

I, the parent or guardian of ____________________________, a child of ______ years of age, permits his/her participation in a program of research named above and being conducted by Isaac Djan Asare.

Signature of Parent or Guardian------------------------------- Date -----------

Please print your name here…………………………………………………………

Signature of Investigator: _________________________________ Date: _________
EVALUATION OF RENAL AND HEPATIC DYSFUNCTION AMONG CHILDREN LESS THAN FIVE YEARS WITH MALARIA IN JASIKAN DISTRICT, VOLTA REGION

Dear respondent,

I am a final year Mphil. student from University of Ghana, legon, Department of Chemical Pathology researching on the effects of malaria on the kidney and liver among children less than five years. You are guaranteed beyond reasonable doubt that all information given will be treated as highly confidential and your answers will never be identified or traced back to you. I will be most appreciative if you partake in this study.

CODE…………………….. DATE……………………

<table>
<thead>
<tr>
<th>General Biodata information</th>
<th>(please tick or write where appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Child’s age</td>
<td>Please indicate below(Years and Months)</td>
</tr>
<tr>
<td></td>
<td>.............................................</td>
</tr>
<tr>
<td>2. Child’s gender</td>
<td>Male □ Female □</td>
</tr>
<tr>
<td>3. Location</td>
<td>Please indicate below</td>
</tr>
<tr>
<td>4. Child’s weight</td>
<td>Please indicate below</td>
</tr>
<tr>
<td>5. Age of Guardian</td>
<td>Please indicate below</td>
</tr>
<tr>
<td>6. Educational status of guardian</td>
<td>None □ Basic □ Secondary □ Tertiary □</td>
</tr>
<tr>
<td>7. Marital status</td>
<td>Cohabitation □ Single □ Married □</td>
</tr>
<tr>
<td>8. Guardian’s Religion</td>
<td>Christian □ Muslim □ Traditional □ Others………………………….</td>
</tr>
</tbody>
</table>

KNOWLEDGE ON MALARIA/ Influence of Health seeking behaviour

9. Within the past six months did your child encounter any episodes of fever? | Yes □ No □
<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Was he/she taken to the hospital?</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>If no, can you please explain the rationale?</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>If Yes how many days did it take before moving to the hospital</td>
<td>Please specify</td>
</tr>
<tr>
<td>13</td>
<td>What drug was he/she given before you left the hospital</td>
<td>Others</td>
</tr>
<tr>
<td>14</td>
<td>Can you please name the type of antimalaria, if your answer is in affirmative in question 13?</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>What causes malaria?</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>What are the signs of malaria?</td>
<td>a) Fever</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Headache</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Feeling cold</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) With a tendency of bask in the sun</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e) General body weakness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>f) Body/joint pains</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g) Vomiting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>h) Abdominal pain/Diarrhoea</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i) Convulsion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>j) Don’t know</td>
</tr>
<tr>
<td>17</td>
<td>What symptoms is your ward experiencing now?</td>
<td>Please specify</td>
</tr>
<tr>
<td>18</td>
<td>Do you have insecticide treated nets?</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>If yes,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Question</td>
<td>Yes</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>19.</td>
<td>Do your child sleep in the Insecticide treated net?</td>
<td>Yes</td>
</tr>
<tr>
<td>21.</td>
<td>If no to 18, why</td>
<td>Please specify..................................</td>
</tr>
<tr>
<td>22.</td>
<td>If no to 19, why</td>
<td>Please specify..................................</td>
</tr>
<tr>
<td>21.</td>
<td>Any laboratory test (Bf for Mp’s) requested and done for your ward?</td>
<td>Yes</td>
</tr>
</tbody>
</table>
APPENDIX D: ETHICAL CLEARANCE

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

Ref. No.: EPRC/DEC/2018

December 5, 2018

Isaac Djan Asare
Dept. Chemical Pathology
SBAHS
UG/Korle-Bu

ETHICAL CLEARANCE

FWA: 000185779 IORG: 0005170 IRB: 00006220

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on November 29th 2018 reviewed and approved your research protocol.

Title of Protocol: “Evaluation of Renal and Hepatic Dysfunction among Children less than five years with Malaria in Jasan District, Volta Region-Ghana”

Principal Investigator: Isaac Djan Asare

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 extension of ethical clearance is valid till December 8, 2019.

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

Signed: [Signature]
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Dept. Chemical Pathology

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University of Ghana http://ugspace.ug.edu.gh