KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

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MALARIA AND ANAEMIA IN PREGNANT AND NON-PREGNANT WOMEN OF CHILD-BEARING AGE AT THE UNIVERSITY HOSPITAL - KNUST, KUMASI

BY

ERIC AGBOLI (B.Sc., Hons.)

AUGUST, 2011

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A DISSERTATION SUBMITTED TO

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DECLARATION

I undertook the experimental work herein described under the supervision of Dr. S.C.K. Tay of the Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology. All references sited in this work have been duly acknowledged.

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(HEAD OF DEPARTMENT) Signature Date

DEDICATION

To my lovely mother Adzotor Kpekpena and my siblings; Emmanuel Agboli, Christiana Agboli and Ernest Agboli for their financial support and encouragement. This is also dedicated to Miss Rosemary Esiawonam Atisu, Mr Mawuli Akorli and my late Grandfather Torgbi Awanya all of Akatsi for their love, encouragement, and kindness. Grandpa may your soul rest in perfect peace.

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DEFINITION OF TERMS

Anaemia

This is a blood condition in which there are too few red blood cells or the red blood cells are deficient in haemoglobin. Haemoglobin level < 11g/dl = anaemic; haemoglobin level ≥ 11g/dl = non-anaemic.

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Antenatal Clinic

It is a specific clinic that takes care of the health needs of pregnant women.

Intermittent Preventive Treatment (IPT)

Drug given to pregnant women at least two doses during the second and third trimester.

Insecticide Treated Net (ITN)

Bed net that has been treated with an insecticide in the last 6 months.

Malaria

It is defined as the presence in the peripheral or venous blood of asexual blood stage of *Plasmodium*, irrespective of species or symptoms.

Multigravidae

A pregnant woman who has had two or more pregnancies.

Parasitaemia

This is defined as presence of malaria parasites in blood films from peripheral circulation as counted per 100 high power fields.

Pregnancy

The state of being with a child and it ranges from the time of conception to delivery of the conceptus.

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Primigavidae

One who is pregnant for the first time.

Sulphadoxine pyrimethamine (SP)

This is the drug given to the pregnant women in an Intermittent Preventive Treatment programme.

Young age

This age was defined as age < 20 years

ABBREVIATION/ACRONYMS

AIDS	-	Acquired Immunodeficiency Syndrome
ANC	-	Antenatal Clinic
BMI	-	Body Mass Index
HIV	-	Human Immunodeficiency Virus
ITN	-	Insecticide Treated Net
ITNs	-	Insecticide Treated Nets
IPT	-	Intermittent Preventive Treatment
IPTs	-	Intermittent Preventive Treatments
KNUST	-	Kwame Nkrumah University of Science and Technology
SP	-	Sulphadoxine Pyrimethamine
WHO	-	World Health Organisation

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ABSTRACT

Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world. This study was conducted to compare the prevalence of malaria and anaemia in pregnant and non-pregnant women of child-bearing age at the University Hospital- KNUST, Kumasi. This is a cross sectional, comparative study conducted from February to December 2010 at the Hospital. Using a systematic method, 380 each of pregnant and non-pregnant women were screened for the study. Blood and stool samples were collected from participants who were referred to the laboratory for routine examination. Pregnant women have higher malaria parasitaemia (12.6 %) compared to 6.6% in non-pregnant women. The species isolated from the pregnant women were *P. falciparum* (85.4%), *P. malariae* (4.2%) and *P. ovale* (10.4%). Among non-pregnant women of child-bearing age, 76% *P. falciparum*, 8% *P. malariae* and 16% *P. ovale* were isolated. Anaemia was high in pregnant women (62.6%) compared to their non-pregnant counterparts (53.2%) and intestinal nematodes were not associated with anaemia in pregnant women. Age of pregnant women was a factor affecting malaria parasitaemia with a significant P-value and OR (P-value = 0.0041, 0R =7.61). Malaria infection was common in nulliparous women, and most of the pregnant women were in their second trimester at the time of screening. Malaria parasitaemia was higher in the primigravidae (14%) and multigravidae recorded the highest anaemia prevalence (67.1%). The highest prevalence of malaria (28.6%) and anaemia (69.0%) were among pregnant women in their third trimester. Pregnant women reporting at the antenatal care were not given intermittent preventive treatment (IPT). There was increased risk of malaria parasitaemia in pregnancy in the use of 'others' (mosquito coils, creams, repellents and insecticide sprays) compared to ITN usage with a significant P-value (OR = 4.17, 95% CI = 1.90-9.19 and P-value = 0.0001 for 'others' and OR = 0.23, 95% CI = 0.24-1.51 and P-value < 0.0001 for ITN). Malaria parasitaemia and anaemia are found to be common medical conditions associated with pregnancy. Pregnant women are more susceptible to malaria and anaemia compared to their non-pregnant counterparts.

Malaria was the major cause of anaemia in both pregnant and non-pregnant women. Efforts should be geared towards the control of malaria and anaemia during pregnancy. Other anaemia causing agents apart from malaria should be investigated in future studies.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

The word "Malaria" was derived from two Italian words, "mal" and "aria", meaning "bad air" because it was first thought that the disease came from fetid marshes (Reiter, 2000). In 1880, scientists discovered the real cause of malaria-a one-cell parasite from the genus *Plasmodium* (Reiter, 2000). Later, it was discovered that the parasite is transmitted from person to person through the bite of the female Anopheles mosquito, which requires blood to nurture her eggs (Reiter, 2000).

Malaria is a serious public health problem particularly in pregnant women in the tropics (Nwonwu *et al.*, 2009). *Plasmodium falciparum* is responsible for the majority of malaria infections that occur in pregnancy as compared to other species of the parasite (Omo-Aghoja *et al.*, 2008). *Plasmodium falciparum* malaria infection in pregnant women may have significant adverse consequences for both mother and child (McGregor, 1984). Malaria is more frequent in pregnant women than in age-matched controls, and in areas of low endemicity such as Southeast Asia, severe or complicated malaria may also occur (McGregor, 1984). There is evidence that severe malaria may also be a significant problem in pregnant women in urban areas in sub-Saharan Africa (Granja *et al.*, 1998).

Despite the tremendous efforts committed to the control of malaria, the most common, serious mosquito-borne disease in the world, it still remains a public health challenge in more than 90 countries, inhabiting about 40% of the world's population (Uneka, 2009). Current estimates of the World Health Organization (WHO) indicate that malaria causes 300-500 million infections per year with 1.5-2.7 million deaths, more than 90% in children under the age of 5 in Africa (Delacollette *et al.*, 2009).

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Parasitaemia of the maternal placental blood is more frequent than parasitaemia of the maternal peripheral blood (Brabin, 1991; McGregor, 1984; Menendez, 1995). This affects 10–34% of all pregnant women and primigravidae are more heavily and more often infected (up to twice as much) than multigravidae (Brabin, 1991; McGregor, 1984; Menendez, 1995). It is also difficult to assess the impact of maternal malaria infection on perinatal and infant mortality. Based on the few studies available, it was estimated that pregnancy-associated malaria was responsible for 3–8% of infant deaths, involving approximately 75,000–200,000 infants every year (Steketee *et al.*, 2001).

Low transmission typically delays the development of immunity and all age groups of a sedentary urban population, rather than just young children and pregnant women alone, would be expected to be at risk of severe complicated malaria (Klinkenberg *et al.*, 2006). Despite these challenges, urban malaria could be readily and cost-effectively controlled, if diagnosis and treatment were focused on the most vulnerable pregnant women and children under five years (Donnelly *et al.*, 2005).

Pregnant women with *Plasmodium falciparum* are prone to complications such as, hypoglycemia, acute pulmonary edema, foetal distress, premature labour, spontaneous abortions and still births (Singh *et al.*, 1999).

Severe anemia predominates as the main feature of severe malaria in areas with high levels of transmission, while hypoglycemia, respiratory failure, and cerebral malaria may predominate in areas with low levels of malaria transmission (Nosten *et al.*, 2004; Whitty *et al.*, 2005). Anaemia in pregnancy is estimated to affect approximately 50% of pregnant women in malaria-endemic countries of Africa (WHO, 1992; WHO/UNICEF/UNU, 2001). It is an important public health problem worldwide (WHO, 1994). WHO estimates that more than half of pregnant women in the World have a haemoglobin level indicative of anaemia (< 11.0gldl), the prevalence may however be as high as 56 or 61% in developing countries (WHO, 1994).

With an estimated 200 million urban residents currently at risk of malaria and a projected doubling of the African urban population by 2030 (UNDP, 2004) due to rapid expansion, greater number of pregnant and non-pregnant women of child-bearing age will be at risk of infection due to increasing urban migration and lack of information (Donnelly *et al.*, 2005). It is therefore important to conduct research to identify risk factors for malaria in pregnant and non-pregnant women (Donnelly *et al.*, 2005).

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1.2 Problem statement

Each year 25 million African women become pregnant in malaria endemic areas (WHO/AFRO, 2004). In sub-Saharan Africa, where 80–90% of the world's malaria cases occur, approximately 19–24 million women are at risk for malaria accompanied by adverse consequences in pregnancy (Guyatt & Snow, 2001).

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The intense malaria transmission conditions found in many parts of tropical Africa, the much lower malaria inoculation rates currently sustained in areas of Southeast Asia and the epidemic outbreaks of malaria occasionally seen in both continents, present highly contrasting patterns of malaria-related mortality (Alles *et al.*, 1998). Despite this well-documented indirect morbidity burden, it is generally assumed that due to the acquisition of significant levels of malaria immunity in areas of stable transmission, parasitaemic pregnant women are rarely symptomatic, and that severe disease or death from malaria is extremely unusual (Nosten *et al.*, 2004).

In southern Ghana, malaria in pregnancy and related morbidity are frequent (Mockenhaupt *et al.*, 2006). However, resistance to sulphadoxine pyrimethamine (SP) is steadily increasing in some areas in sub-Saharan Africa, and the available arsenal of alternative tools for malaria control in pregnancy is very limited due to financial constraints (Steketee & Mutabingwa, 1999). In Ghana, SP achieves cure rates within 28 days of follow-up of 14% and 11% in children and pregnant women with uncomplicated malaria, respectively (Tagbor *et al.*, 2006).

In randomized-controlled trials conducted to determine the impact of insecticide treated nets (ITNs) in pregnancy, covering a wide spectrum of malaria endemicity ranging from unstable-low to high and markedly seasonal malaria transmission, ITNs significantly reduced malaria parasitaemia and maternal anemia and increased birth weight, in areas with the lowest and most seasonal transmission (Browne *et al.*, 2001). No impact was observed in areas with more intense transmission (Browne *et al.*, 2001).

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Malaria, apart from its public health importance, is also of economic significance (Gallup & Sachs, 2001):

- Huge cost burden on endemic countries; spending by government on maintaining health facilities and health care infrastructure, publicly managed vector control, education and research.
- The cost of treatment and prevention; ITNs, doctor's fees, anti-malarial drugs, transport to health facilities, support for the patient and sometimes accompanying family member during hospital stays.
- Time spent seeking treatment or looking after sick ones.
- Indirect cost such as lost productivity as a result of morbidity and mortality; cost of lost workdays or absenteeism from formal employment and the value of unpaid work done in the home by both men and women.
- The cost of malaria in terms of human suffering is underestimated; in the case of death, the indirect cost includes the discounted future lifetime earnings of those who die (Gallup & Sachs, 2001).

Approximately $ 12 billion is lost annually through malaria (Gallup & Sachs, 2001). Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria (Gallup & Sachs, 2001). Economists believe that malaria is responsible for a 'growth penalty' up to 1.3% per year in some African countries (Gallup & Sachs, 2001). Compounded over the years, this penalty leads to substantial differences in gross domestic product (GDP) between countries with and without malaria and severely restrains economic growth of the entire region (Gallup & Sachs, 2001). Between 1965 and 1990, countries in which large proportion of the population lived in regions with *Plasmodium falciparum* malaria experienced an average growth in per-capita GDP of 0.4% per year, whereas average growth in other countries was 2.3% per year (Gallup & Sachs, 2001). Economic activities such as tourism are affected.

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The cost of treating malaria episode is ever increasing (Gallup & Sachs, 2001). Studies were done on the prevalence of malaria in pregnancy, but detailed study was not done on malaria parasitaemia in pregnant women compared to non-pregnant women of child-bearing age with gynaecological complaints. The gynaecological aspect of the study made it unique as compared to previous studies on malaria in pregnancy. The present study will be another source of information for the fight against malaria not only during pregnancy but also in non-pregnant women of child-bearing age.

1.3 Justification

The epidemiology and impact of malaria during pregnancy have been conducted in regions with high endemicity for malaria where *Plasmodium falciparum* is the major cause of malaria (Brabin, 1991). Pregnant women are 4–12 times more likely to be parasitemic compared with other adults (non-pregnant women of child-bearing age) (Brabin, 1991). No study on *P. malariae and P. ovale* morbidity in pregnant women and non-pregnant women of child-bearing age compared to *P. falciparum* is reported in Ghana. Moreover, the epidemiology of *P. falciparum* malaria in pregnant women and non-pregnant women of child-bearing age in regions of low malaria endemicity, and malaria caused by *P. ovale and P. malariae* infections remains unclear and requires further description to develop effective preventive and control programmes and budgetary allocations for pregnant women and non-pregnant women of child-bearing age.

Efforts to control malaria have been largely directed at controlling the vector and developing effective preventive and therapeutic drugs (WHO, 2002). However, these efforts and the hopes of malaria control and eradication are seriously challenged by the sad reality of 1.5 million to 2.7 million lives that succumb to malaria worldwide each year (WHO, 2002). New approaches are therefore needed to curtail this challenge.

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Little information is currently available on the epidemiology and impact of malaria during pregnancy and in non-pregnant women of child-bearing age in the Ashanti Region of Ghana, which makes it difficult to develop effective preventive and control strategies specific for pregnant and non-pregnant women of child-bearing age.

In 145 pregnant and 79 non-pregnant women, malaria infection was recorded in Jabalpur district of India (Singh *et al.*, 1995). *Plasmodium falciparum* was the most prevalent species accounting for 72% of the total malaria infection in pregnant women while, in non-pregnant women it accounted for 58% (Singh *et al.*, 1995). These results emphasize the need to target malaria control for these groups of women (pregnant and non-pregnant).

Therefore, there is the need to conduct this study to provide a preliminary comparative description of the prevalence of malaria and anaemia in pregnant and non-pregnant women at the University Hospital KNUST, and the risk factors associated with clinical malaria. Due to lack of a study on malaria and anaemia in pregnant and non-pregnant women of child-bearing age, the incidence of malaria and anaemia in pregnant women compared with their non-pregnant counterparts is not assessed at the University Hospital KNUST.

1.4 Hypothesis

This study is based on the hypothesis that malaria parasitaemia and anaemia are high in pregnant women as compared to their non-pregnant counterparts and that age, stage of pregnancy, gravidity, and parity are independent determinants of prevalence of malaria and anaemia presenting at the University Hospital KNUST.

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1.5 Research questions

The purpose of the study was to answer the following research questions:

- What species of *Plasmodium* is the main cause of malaria in the participants?
- What is responsible for anaemia in the pregnant women?
- What effects do ITN usage and IPT have on malaria parasitaemia?
- At which stage of pregnancy is malaria parasitaemia said to be high?

These questions are to be resolved with much confidence in the present study and relevant recommendations given to prevent malaria infection in pregnant and non-pregnant women.

1.6 Main objective

To determine malaria parasitaemia and anaemia in pregnant and non-pregnant women of child-bearing age at the University Hospital, KNUST, Kumasi.

1.7 Specific objectives

- To determine the prevalence of malaria in pregnancy in relation to parity and gravidity.
- To monitor malaria parasitaemia and anaemia at different stages of pregnancy.
- To investigate the effect of the use of IPT and ITN on malaria parasitaemia in pregnant women.
- To investigate the effect of intestinal helminthes on anaemia in pregnant women.
- To determine the *Plasmodium* species associated with malaria cases using RDT.

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1.8 Assumptions

1. It is hereby assumed that all the pregnant women attending ANC were infected with malaria.
2. It is also assumed that all the malaria cases presented at University Hospital ANC were through female anopheles mosquito bites.
3. Susceptibility of pregnant women to malaria infection is higher as compared to the non-pregnant women of child-bearing age who have not given birth during the study period.
4. Anaemia is caused by malaria and intestinal nematodes in pregnant women.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The history of malaria, an ancient disease

The history of malaria predates humanity, as this ancient disease evolved before humans did (Reiter, 2000). As malaria remains a major public health problem, causing more than 225 million clinical cases each year (Phillips, 2010), killing about 781,000 people each year according to the World Health Organisation's 2010 World Malaria Report, 2.23% of deaths worldwide (WHO, 2010b).

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2.1.1 Origin and early history

Human malaria likely originated in Africa and has coevolved along with its definitive hosts, mosquitoes and intermediate hosts, non-human primates (Poinar, 2005). The first evidence of malaria parasites was found in mosquitoes preserved in amber from the Paleogene period that were approximately 30 million years old (Poinar, 2005). Malaria may have been a human pathogen for the entire history of the species (Hayakawa *et al.*, 2008; Joy *et al.*, 2003). Close relatives of the human malaria parasites remain common in chimpanzees, the closest evolutionary relative of modern humans (Martin *et al.*, 2005; Roy & Irimia, 2008).

Malaria started having a major impact on human survival about 10,000 years ago which coincides with the introduction of agriculture (Neolithic revolution) (Hempelmann *et al.*, 2009).

The consequence was natural selection for sickle-cell disease, thalassaemias, glucose-6-phosphate dehydrogenase deficiency, ovalocytosis, elliptocytosis and loss of the Gerbich antigen (glycophorin C) and the Duffy antigen on the erythrocytes because such blood disorders confer a selective advantage against malaria infection (balancing selection) (Canali, 2008). The three major types of inherited genetic resistance (sickle-cell disease, thalassaemias, and glucose-6-phosphate dehydrogenase deficiency) were present in the Mediterranean world by the time of the Roman Empire, about 2000 years ago (Sallares *et al.*, 2004).

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The name malaria, derived from 'mal'aria' (bad air in Medieval Italian) was probably first used by Leonardo Bruni in a publication (Bruni, 2004). Malaria was once common in most of Europe and North America, where it is now for all purposes non-existent (Knottnerus, 2002). The coastal plains of southern Italy, for example, fell from international prominence (the Crusaders going by sea to the Holy Land took ship at Bari) when malaria expanded its reach in the sixteenth century (Knottnerus, 2002).

At roughly the same time, in the coastal marshes of England, mortality from "marsh fever" or "the ague" (from Latin "febris acuta") was comparable to that in sub-Saharan Africa today (Knottnerus, 2002). William Shakespeare was born at the start of the especially cold period that climatologists called the "Little Ice Age", yet he was aware enough of the ravages of the disease to mention it in eight of his plays (Reiter, 2000).

Throughout history the most critical factors in the spread or eradication of the disease has been human behaviour (shifting population centers, changing farming methods etc.) and living standards (Breman, 2001). Precise statistics are unknown because many cases occur in rural areas where people do not have access to hospitals or the means to afford health care (Breman, 2001). As a consequence, the majority of cases are undocumented (Breman, 2001). Poverty has been and remains a reason for the disease to remain today while it has undergone a decline in other locations (Worrall *et al.*, 2005).

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2.1.2 Discovery of malaria parasite (1880)

The causal relationship of pigment to the parasite was established in 1880, when the French physician Charles Louis Alphonse Laveran, working in the military hospital of Constantine, Algeria, observed pigmented parasites inside the red blood cells of people suffering from malaria (Cox, 2010). He also witnessed the events of exflagellation and became convinced that the moving flagella were parasitic microorganisms (Cox, 2010). He noted that quinine removed the parasites from the blood. Laveran called this microscopic organism *Oscillaria malariae* and proposed that malaria was caused by this protozoan (Cox, 2010).

2.1.3 Differentiation of species of malaria (1886)

In 1885 Ettore Marchiafava, Angelo Celli and Camillo Golgi studied the reproductive cycles in human blood (Golgi cycles) (Smith & Sanford, 1985).

Golgi observed that all parasites present in the blood divided almost simultaneously at regular intervals and that division coincided with attacks of fever (Smith & Sanford, 1985). Golgi also recognized that the three types of malaria are caused by different protozoan organisms (Smith & Sanford, 1985). Marchiafava and Celli called the new microorganism *Plasmodium* (Smith & Sanford, 1985).

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Camillo Golgi, an Italian neurophysiologist, established that there were at least two forms of the disease, one with tertian periodicity (fever every other day) and one with quartan periodicity (fever every third day). He also observed that the forms produced differing numbers of merozoites (new parasites) upon maturity and that fever coincided with the rupture and release of merozoites into the blood stream. He was awarded a Nobel Prize in Medicine for his discoveries in neurophysiology in 1906 (http://www.cdc.gov/malaria/history/index.htm).

2.1.4 Naming of human malaria parasites (1890, 1897)

The Italian investigators Giovanni Batista Grassi and Raimondo Filetti first introduced the names *Plasmodium vivax* and *P. malariae* for two of the malaria parasites that affect humans in 1890. Laveran had believed that there was only one species, *Oscillaria malariae*. An American, William H. Welch, reviewed the subject and, in 1897, he named the malignant tertian malaria parasite, *P. falciparum*.

There were many arguments against the use of this name; however, the use was so extensive in the literature that retaining the name given by Laveran was no longer thought possible. In 1922, John William Watson Stephens described the fourth human malaria parasite, *P. ovale* (http://www.cdc.gov/malaria/history/index.htm).

2.1.5 Discovery that mosquitoes transmit malaria parasites (1897-1898)

On August 20th, 1897, Ronald Ross, a British officer in the Indian Medical Service, was the first to demonstrate that malaria parasites could be transmitted from infected patients to mosquitoes. Working with bird malaria, Ross showed that mosquitoes could transmit malaria parasites from bird to bird (http://www.cdc.gov/malaria/history/index.htm). This initiated a sporogonic cycle (the time interval during which the parasite developed in the mosquito) (http://www.cdc.gov/malaria/history/index.htm). Thus, the problem of malaria transmission was solved (http://www.cdc.gov/malaria/history/index.htm). Ross was awarded the Nobel Prize in 1902 (http://www.cdc.gov/malaria/history/index.htm).

2.1.6 Discovery of the transmission of the human malaria parasites, *Plasmodium* (1898-1899)

Giovanni Batista Grassi leading a team of Italian investigators (1898), which included Amico Bignami and Giuseppe Bastianelli, collected *Anopheles claviger* mosquitoes and fed them on malarial patients. The complete sporogonic cycle of *Plasmodium falciparum, P. vivax,* and *P. malariae* was demonstrated as the mosquitoes were fed on the malarial patients.

For confirmation of the role of mosquito in transmitting malaria in 1899, mosquitoes which were fed on malarial patient in Rome were sent to London where they fed on two volunteers, both of whom developed benign tertian malaria (http://www.cdc.gov/malaria/history/index.htm)

2.1.7 Early research and treatment

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For thousands of years, traditional herbal remedies have been used to treat malaria (Willcox & Bodeker, 2004). Hippocrates (460–370 BC), the "father of medicine", related the presence of intermittent fevers with climatic and environmental conditions and classified the fever according to periodicity: tritaios pyretos / febris tertian, and tetrataios pyretos / febris quartana (every fourth day) (Pappas *et al.*, 2008).

Qinghao (*Artemisia annua*), a herbal remedy, was first described by Ge Hong (283–343 AD) as an effective medication in the 4th century Chinese manuscript *Zhou hou bei ji fang*, usually translated as "Emergency Prescriptions kept in one's Sleeve" (Wright *et al.*, 2010). Quinine (Kinine), a toxic plant alkaloid, was long used by the Quechua Indians of Peru to reduce the shaking effects caused by severe chills in the Andes (Guay, 2008). The use of the "fever tree" bark was introduced into European medicine by Jesuitical missionaries (Jesuit's bark) (Kaufman & Ruveda, 2005).

2.1.8 Chloroquine (resochin) (1934, 1946)

Chloroquine was discovered by a German, Hans Andersag, in 1934 at Bayer I.G. Farbenindustrie A.G. laboratories in Eberfeld, Germany. He named his compound resochin (http://www.cdc.gov/malaria/history/index.htm). Through a series of lapses and confusion brought about during the war, chloroquine was finally recognized and established as an effective and safe antimalarial in 1946 by British and U.S. scientists (http://www.cdc.gov/malaria/history/index.htm).

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2.1.9 Dichloro-diphenyl-trichloroethane (DDT) (1939)

A German chemistry student, Othmer Zeidler, synthesized DDT in 1874, for his thesis. The insecticidal property of DDT was not discovered until 1939 by Paul Müller in Switzerland (http://www.cdc.gov/malaria/history/index.htm). Various militaries in WWII (World War II) utilized the new insecticide initially for louse-borne typhus. DDT was used for malaria control at the end of WWII after it had proven effective against malaria-carrying mosquitoes by British, Italian, and American scientists. Müller won the Nobel Prize for Medicine in 1948 (http://www.cdc.gov/malaria/history/index.htm).

2.2 Epidemiology

Malaria is a mosquito-borne infectious disease caused by a eukaryotic protist of the genus *Plasmodium* (www.en.wikipedia.org/wiki). Five species of the *Plasmodium* parasite can infect humans; the most serious forms of the disease are caused by *Plasmodium falciparum* (www.en.wikipedia.org/wiki).

Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease in humans that is not generally fatal (www.en.wikipedia.org/wiki). A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques but can also infect humans (Fong *et al.*, 1971; Singh *et al.*, 2004).

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Malaria causes about 250 million cases of clinical disease and approximately one million deaths annually (WHO, 2008). The vast majority of cases occur in children under 5 years old; pregnant women are also especially vulnerable (Greenwood *et al.*, 2005). Despite efforts to reduce transmission and increase treatment, there has been little change in which areas are at risk of this disease since 1992 (Hay *et al.*, 2004). Indeed, if the prevalence of malaria remains on its present upwards course, the death rate could double in the next twenty years (Breman, 2001). Precise statistics are unknown because many cases occur in rural areas where people do not have access to hospitals or the means to afford health care. As a consequence, the majority of cases are undocumented (Breman, 2001).

Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa; however, it is in Sub-saharan Africa where 85– 90% of malaria fatalities occur (Layne, 2007) (Figure 1&2). The geographic distribution of malaria within large regions is complex, and malaria-afflicted and malaria-free areas are often found close to each other (Greenwood & Mutabingwa, 2002).

In drier areas, outbreaks of malaria can be predicted with reasonable accuracy by using rainfall data (Grover-Kopec *et al.*, 2005). Malaria is more common in rural areas than in cities; this is in contrast to dengue fever where urban areas present the greater risk (Van Benthem *et al.*, 2005) while the cities of Vietnam, Laos and Cambodia are essentially malaria-free. The disease is present in many rural regions (Trung *et al.*, 2004). In contrast, malaria is present in both rural and urban areas in Africa, though the risk is lower in the larger cities (Keiser *et al.*, 2004).

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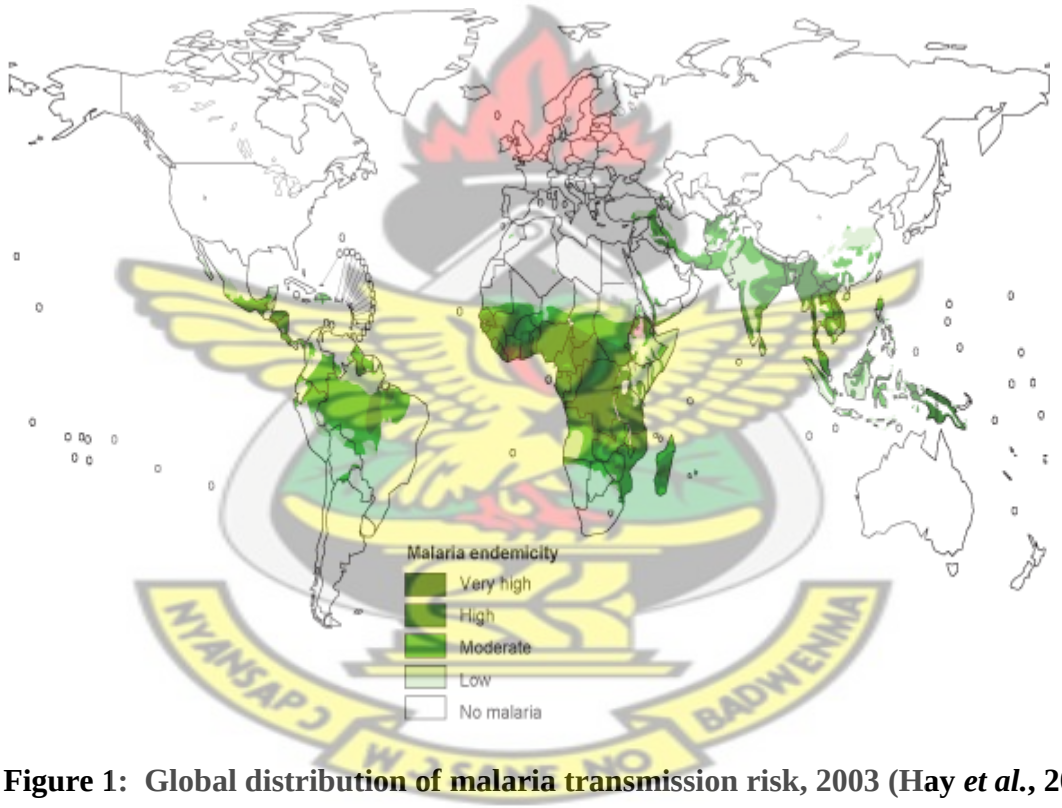

Figure 1: Global distribution of malaria transmission risk, 2003 (Hay *et al.*, 2004).

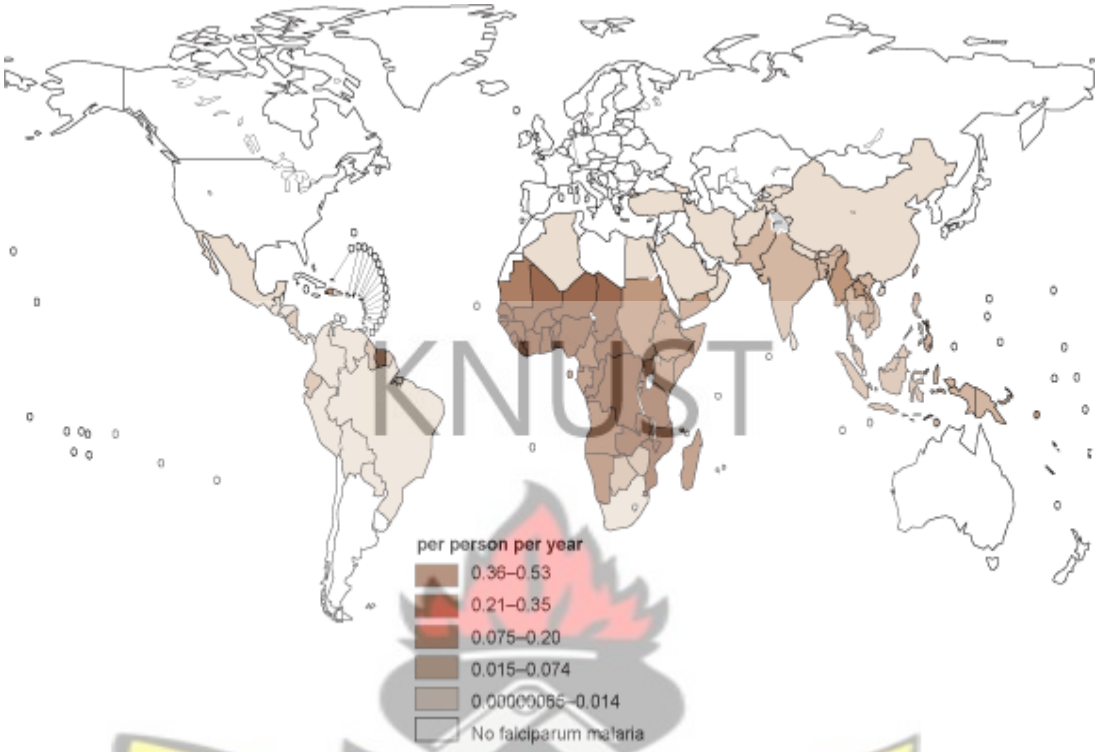

Figure 2: Estimated incidence of clinical *P. falciparum* episodes resulting from local transmission, country level averages, 2004 (Korenromp, 2004).

Malaria is widespread in tropical and subtropical regions, including South America, Asia, and Africa (CDC, 2010). Each year, there are approximately 350–500 million cases of malaria (CDC, 2010). Malaria kills between one to three million people, the majority of whom are young children in Sub-saharan Africa (Snow *et al.*, 2005). Each year 25 million African women become pregnant in malaria endemic areas (WHO/AFRO, 2004). In most of these settings malaria transmission is stable and *Plasmodium falciparum* is predominant (Steketee *et al.*, 2001).

Approximately 30 million pregnant women are exposed to the risk of malaria infection every year (WHO/UNICEF, 2003). Anaemia in pregnancy is estimated to affect approximately 50% of pregnant women in malaria-endemic countries of Africa (WHO, 1992; WHO/UNICEF/UNU, 2001).

Malaria infection during pregnancy is an enormous public health problem, with substantial risks for the mother, her fetus and the neonate (McGregor, 1984). In areas of low transmission of *Plasmodium falciparum*, where levels of acquired immunity are low, pregnant women are susceptible to episodes of severe malaria, which can result in stillbirths, spontaneous abortion or can cause death of the mother while giving birth (Luxemburger *et al.*, 1997). In areas of high transmission of *P. falciparum*, where levels of acquired immunity tend to be high, pregnant women are susceptible to asymptomatic infection, which can result in maternal anaemia and placental parasitaemia, both of which can subsequently lead to low birth weight (Steketee *et al.*, 1996).

In many African countries where malaria is holo-endemic, non-pregnant female adults eventually achieve a significant level of immunity against malaria (Nwonwu *et al.*, 2009). However, during pregnancy, these women experience considerable decline in their levels of immunity to malaria (Nwonwu *et al.*, 2009). Several studies have reported that first and second pregnancies are associated with a higher prevalence of malaria parasitaemia in the first half of pregnancy especially in women living in endemic malarious areas (Brabin, 1983; Okwa, 2003).

During the late 1930s, the Northeast Region of Brazil was invaded by *Anopheles gambiae* and a severe malaria outbreak, with a 13% fatality rate in a largely immune-naïve population, astonished Brazilian malariologists and health authorities. Because of the shipping traffic between Brazil and Senegal at that time, it was assumed that the invader came from this African region, probably in French warships travelling about 70 hours from Dakar to Natal to conduct meteorological studies preparatory of the transatlantic flights to be done by the future commercial companies (Deane, 1992).

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The contribution of malaria to morbidity and mortality among non-pregnant women in Africa has been a subject of academic interest, political advocacy, and speculation (Singh *et al.*, 1995). An empirical approach to define *Plasmodium falciparum* transmission limits across the continent was done to estimate mortality, morbidity, and disability due to malaria among Africa's non-pregnant population (Snow *et al.*, 1999). The results indicated that among populations exposed to stable endemic malaria in sub-Saharan Africa, approximately 987,466 people might have died in 1995 due to malaria infection (Singh *et al.*, 1995). On the other hand, over 207.5 million clinical attacks of malaria may have occurred (Snow *et al.*, 1999).

The endemicity of malaria is defined traditionally in terms of palpability of the spleen or parasite rates in children aged between 2 and 9 years (Cook, 1996), as follows:

- Hypoendemic: spleen rate or parasite rate of 0-10%
- Mesoendemic: spleen or parasite rate of 10-50%

- Hyperendemic: spleen or parasite rate of 50-75%; adult spleen rate is also high
- Holoendemic: spleen or parasite rate of over 75%; but adult spleen rate low and parasite rates in the first year of life are high (Cook, 1996).

2.3 Life cycle of the *Plasmodium* parasite

There are two phases in the life cycle: the sexual cycle, which occurs primarily in mosquitoes, and the asexual cycle, which occurs in humans, the intermediate hosts (Beaver & Jung, 1985; Cook, 1996). The sexual cycle is called sporogony because sporozoites are produced, and the asexual cycle called schizogony because schizonts are developed (Beaver & Jung, 1985; Cook, 1996).

The life cycle in humans begins with the introduction of sporozoites into the blood from the saliva of the biting female *Anopheles* mosquito preparatory to taking blood meal (Beaver & Jung, 1985). The sporozoites are taken up by hepatocytes within 30 minutes (Beaver & Jung, 1985). This "exo-erythrocytic" phase consists of cell multiplication and differentiation into merozoites. *P. vivax* and *P. ovale* produce latent forms in the liver called hypnozoites, which cause relapses seen with vivax and ovale malaria (Beaver & Jung, 1985; Cook, 1996).

Merozoites released from the liver cells infect red blood cells (Beaver & Jung, 1985; Cook, 1996). During this erythrocytic stage, the *Plasmodium* differentiates into a ring-shaped trophozoite, which grows into an amoeboid form, and then into a schizont filled with merozoites (Beaver & Jung, 1985; Cook, 1996).

Upon release, merozoites infect other erythrocytes (Beaver & Jung, 1985; Cook, 1996). This cycle is repeated at regular intervals typically for each species (Beaver & Jung, 1985; Cook, 1996). This periodic release of merozoites causes the typical recurrent symptoms of fever, chills, and sweats seen in malaria (Beaver & Jung, 1985; Cook, 1996).

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The sexual cycle begins in the human red blood cell when some merozoites develop into male and others into female gametocytes (Beaver & Jung, 1985; Cook, 1996). The gametocytes are ingested by a female *Anopheles* mosquito during a blood meal to continue the cycle. This is shown in the figure below (Beaver & Jung, 1985; Cook, 1996). Differentiation of the gametocytes within the gut of the mosquito produces either a female macrogamete or 8 spermlike male microgametes (Beaver & Jung, 1985; Cook, 1996). Fertilization occurs to produce a diploid zygote that differentiates into a motile ookinete that burrows into the gut wall of the mosquito (Beaver & Jung, 1985; Cook, 1996). It develops into an oocyst within which many haploid sporozoites are found (Beaver & Jung, 1985; Cook, 1996). The sporozoites are released from the gut wall, migrate to the salivary glands, and now are ready to complete the cycle at the mosquito's next blood meal (Beaver & Jung, 1985; Cook, 1996). In malaria contracted by parenteral inoculation such as blood transfusion, the pre-erythrocytic stage is bypassed and parasite development proceeds directly in the erythcytic stage (Beaver & Jung, 1985; Cook, 1996).

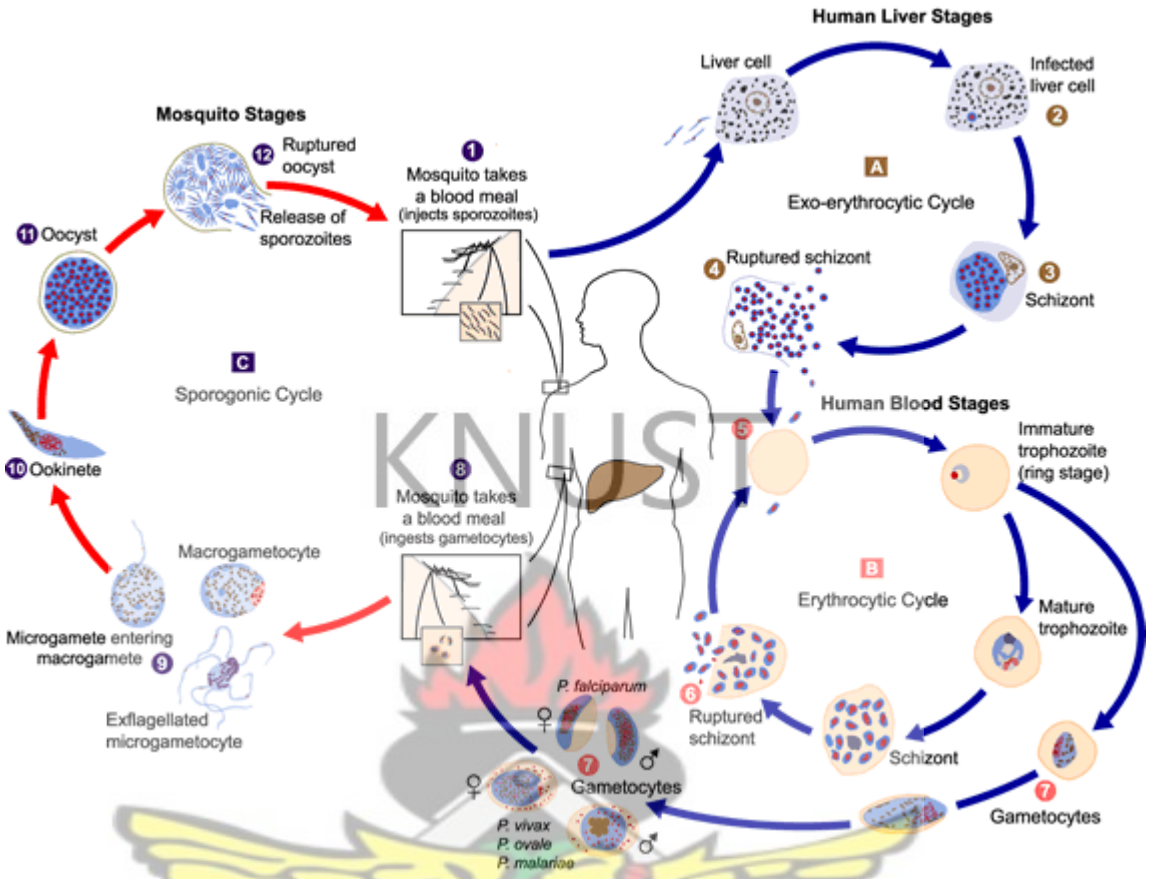

Figure 3: Life cycle of *Plasmodium falciparum*

Apart from female *Anopheles* mosquitoes, there are other ways of contracting malaria. These include the following:

- ***Blood transfusion (Transfusion malaria):*** This is fairly common in endemic areas. Most infections occur in cases of transfusion of blood stored for less than 5 days and it is rare in transfusions of blood stored for more than 2 weeks (http://www.malariasite.com/malaria/Transmission.htm). Frozen plasma is not known to transmit malaria (http://www.malariasite.com/malaria/Transmission.htm).

- ***Mother to the growing fetus (Congenital malaria):*** Intrauterine transmission of infection from mother to child is well documented. Placenta becomes heavily infested with the parasites (http://www.malariasite.com/malaria/Transmission.htm). Congenital malaria is more common in first pregnancy, among non - immune populations (http://www.malariasite.com/malaria/Transmission.htm).
- ***Needle stick injury:*** Accidental transmission can occur among drug addicts who share syringes and needles (http://www.malariasite.com/malaria/Transmission.htm).

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2.4 Pathological and clinical findings

The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhoea, nausea, vomiting, anorexia, and pruritus (Looareesuwan *et al.*, 1999).

The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarials and compromise the quality of care for patients with non-malarial fevers in endemic areas (McMorrow *et al.*, 2008; Mwangi *et al.*, 2005). Most of the pathologic findings of malaria are due to the destruction of erythrocytes, the liberation of parasite and erythrocyte material into circulation, and the host reaction to these (Beaver & Jung, 1985). The spleen also sequesters and destroys many erythrocytes, leading to sinusoidal congestion, coupled with hyperplasia of lymphocytes and macrophages (Cook, 1996).

P. falciparum infection is also characterized by occlusion of capillaries with aggregates of parasitized red cells (Cook, 1996). This leads to life-threatening hemorrhage and necrosis, particularly in the brain (cerebral malaria). Extensive hemolysis and renal damage may ensue, with resulting haemoglobinuria (Cook, 1996). The resultant dark urine of patient has given rise to the term "blackwater fever" (Cook, 1996).

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Malaria presents with an abrupt onset of fever (up to 41^{o}C) and chills, with an accompanying headache, myalgia, and arthralgia, two weeks after an infective mosquito bite (Cook, 1996). These signs and symptoms are the result of endotoxin-like material release when sporozoites rupture, which induces activation of the cytokine cascade (Cook, 1996). First, tumor necrosis factor (TNF), and then interleukin (IL)-1 are produced, which in turn induce the release of other pro-inflammatory cytokines including IL-6 and IL-8. These are responsible for the fever and myalgia associated with malaria (Cook, 1996).

There is usually a concomitant anorexia, nausea, and at times, vomiting. Some patients may experience abdominal pain (Levinson & Jawetz, 1995). The timing of the fever cycle is 72 hours (every fourth day) for *P. malariae*, hence the term quartan malaria; and 48 hours (every third day) for the other plasmodia, hence the term tertian malaria (Levinson & Jawetz, 1995). Drenching sweats follow the fever (Levinson & Jawetz, 1995). Tertian malaria is subdivided into malignant malaria, cause by *P. falciparum*, and benign malaria, caused by *P. vivax* and *P. ovale* (Levinson & Jawetz, 1995).

P. falciparum can infect red cells of all ages causing a high level of parasitaemia. Contrary, *P. vivax* infect only reticulocytes, with *P. malariae* infecting only mature red cells, causing only mild parasitaemia. Untreated, malaria especially one caused by *P. falciparum* is potentially life-threatening due to extensive cerebral and renal damage (Cook, 1996).

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In endemic areas, clinical episodes of malaria are more frequent and more severe during pregnancy and mortality rate is higher among them as compared to non-pregnant women (Ramsay, 2003). Pregnant women are twice as likely to become infected with *P. falciparum* malaria as non-pregnant women living under the same conditions due to physiological changes and suppressed immunity during pregnancy (Lindsay *et al.*, 2000).

Anaemia is more frequent in pregnant women, and more pronounced in primigravidae than in multigravidae (Fleming, 1989; Shulman *et al.*, 1996).

Anaemia is usually multifactorial in origin and although malaria is an important contributor, nutritional deficiencies (iron and folate), other infectious diseases (hookworm, schistosomiasis and HIV) and genetic red blood cell disorders (sickle cell and thalassaemias) are other important contributing factors (van den Broek, 1998). Malaria may cause anaemia through a number of different mechanisms including excess removal of non-parasitized erythrocytes, through auto-immune destruction of parasitized red cells, and impaired erythropoiesis as a result of bone marrow dysfunction (Ekvall, 2003).

Most studies show a strong association between malarial infection of the placenta or peripheral blood and haemoglobin levels, confirming that this is a major cause of anaemia, even when other factors are present (Matteelli *et al.*, 1994; Shulman *et al.*, 1996). However, in a comprehensive review of all studies published between 1985 and 2000, it was estimated that maternal anaemia contributed to 7–18% of LBW (low birth weight) and to 25% of total infant mortality (Steketee *et al.*, 2001).

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2.5 Complications of malaria

P. vivax, *P. ovale*, and *P. malariae* malaria are relatively benign. *P. falciparum* malaria on the other hand is prone to produce serious complications (Beaver & Jung, 1985). These complications, although rare, are worth noting since they could be life-threatening. Malaria complications could be attributed to:

- Cytokine toxicity from cytokines induced by the parasites
- Sequestration, the process whereby erythrocytes containing mature forms of *P. falciparum* adhere to microvascular endothelium, and thus disappear from circulation
- Cytoadherence resulting from anchoring of *P. falciparum* erythrocyte membrane protein (PfEMP)-1 knobs to accretions of parasite-derives histidine-rich protein.
- Vascular endothelial ligands that bind to parasitized erythrocytes
- Resetting, whereby infected erythrocytes adhere to uninfected erythrocytes
- Reduced deformability of erythrocytes due to reduced membrane fluidity, increasing sphericity, and the enlarging and relatively rigid intraerythrocytic parasite

- Generalized increase in systemic vascular permeability (Cook, 1996).

The signs and symptoms of malaria complications vary depending on the organs most infected. Any of them qualifies malaria to be characterized as Severe Malaria, and they include:

1. Cerebral malaria
2. Anaemia
3. Hypoglycaemia
4. Renal failure
5. Pulmonary oedema or adult respiratory distress syndrome
6. Fluid space and electrolyte imbalance or circulatory collapse
7. Coagulopathy and thrombocytopenia
8. Blackwater fever
9. Spleen enlargement
10. Gastrointestinal Dysfunction
11. Liver Dysfunction
12. Metabolic Dysfunction, and
13. Bacterial Superinfection (Cook, 1996; Levinson & Jawetz, 1995).

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2.6 Diagnosis of malaria

2.6.1 Giemsa staining technique

Giemsa stain, named after Gustav Giemsa, an early malariologist, is used for the histopathological diagnosis of malaria and other parasites (Shapiro & Mandy, 2007).

Giemsa stain is also a differential stain (Shapiro & Mandy, 2007). It can be used to study the adherence of pathogenic bacteria to human cells. It differentially stains human and bacterial cells purple and pink respectively (Shapiro & Mandy, 2007). Giemsa stain is used to differentiate nuclear and/or cytoplasmic morphology of platelets, RBCs, WBCs, and parasites (Garcia, 2001; NCCLS, 2000). The most dependable stain for blood parasites, particularly in thick films, is Giemsa stain containing azure B (Garcia, 2001; NCCLS, 2000). Liquid stock is available commercially. The stain must be diluted for use with water buffered to pH 6.8 or 7.0 to 7.2, depending on the specific technique used (Garcia, 2001; NCCLS, 2000). Either should be tested for proper staining reaction before use. The stock is stable for years, but it must be protected from moisture because the staining reaction is oxidative (Garcia, 2001; NCCLS, 2000). Therefore, the oxygen in water will initiate the reaction and ruin the stock stain. The aqueous working dilution of stain is good only for 1 day (Garcia, 2001; NCCLS, 2000). It is a mixture of methylene blue and eosin. The stain is usually prepared from commercially available Giemsa powder.

It is specific for the phosphate groups of DNA and attaches itself to regions of DNA where there are high amounts of adenine-thymine bonding. Giemsa stain is used in Giemsa banding, commonly called G-banding, to stain chromosomes and often used to create an idiogram (Shapiro & Mandy, 2007). It can identify chromosomal aberrations such as translocations and interchanges (Shapiro & Mandy, 2007).

Conventionally, malaria is diagnosed by microscopic examination of peripheral blood for asexual stages of plasmodia, using thick and thin Giemsa-stained smears.

Whereas thick film is used to identify the presence of parasites, the thin film is used for species identification (Levinson & Jawetz, 1995). The thick film is approximately 30 times more sensitive than the thin film (Cook, 1996). Smears from intradermal blood could also be used and may contain more mature forms of *P. falciparum* than peripheral blood and so is slightly more sensitive (Cook, 1996).

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2.6.2 Rapid diagnostic tests (RDTs)

Recently, rapid diagnostic tests (RDTs) are used to diagnose malaria based on the principle of immunochromatography which relies on migration of liquid across the surface of a nitrocellulose membrane (Cook, 1996; Hanscheid *et al.*, 2002). The RDTs have been developed in different test formats like the dipstick, strip, card, pad, well, or cassette. These immunochromatographic tests are based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen targets (Cook, 1996; Hanscheid *et al.*, 2002).

Currently, immunochromatographic tests can target the histidine-rich protein 2 of *P. falciparum*, a pan-malarial *Plasmodium* aldolase, and parasite specific lactate dehydrogenase (Cook, 1996; Hanscheid *et al.*, 2002). Histidine-rich protein 2 of *P. falciparum* (PfHRP2) is a water soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the red cell membrane surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy (Cook, 1996; Hanscheid *et al.*, 2002). *Plasmodium* aldolase is an enzyme of the parasite glycolytic pathway expressed by the blood stages of *P. falciparum* as well as the non-fa1ciparum malaria parasites (Cook, 1996; Hanscheid *et al.*, 2002).

Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and it is present in and released from the parasite infected erythrocytes. It has been found in all four human malaria species, and different isomers of pLDH for each of the four species exist (Cook, 1996; Hanscheid *et al.*, 2002). The pLDH test is formatted to detect a parasitemia of >100 to 200 parasites/µL and some of the PfHRP2 tests are said to detect asexual parasitemia of >40 parasites/µL and giving rapid results (15 to 20 min). The performance of the procedures does not require laboratory, electricity, extensive training or equipment to perform or to interpret the results. The RDTs are expensive, their sensitivities and specificities are variable, and their vulnerability to high temperatures and humidity is an important constraint (Cook, 1996; Hanscheid *et al.*, 2002).

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2.6.3 DNA and RNA detection

The presence of DNA and RNA in malaria parasites, as opposed to red cells, allows its visualization with UV light microscopy when stained with fluorescent dyes (Hanscheid *et al.*, 2002). Polymerase chain reaction (PCR) using, as primers, portions of known parasite DNA sequences, could be applied in malaria diagnosis. This is known to be the most sensitive and specific method to detect malaria parasite, and has acknowledged value in research settings (Hanscheid *et al.*, 2002).

2.6.4 Automated detection

A new generation of automated analyzers that incoporated flow-cytometric principles are currently employed in many haematological laboratories for routine full blood counts (FBC). These provide a novel way to diagnose malaria by automated detection of Hemozoin during FBC analysis (Hanscheid *et al.*, 2000).

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2.6.5 Aspirate/Biopsy

The diagnosis of cerebral malaria could be confirmed post mortem via microscopic examination of a brain smear of grey matter, obtained through a needle aspirate or biopsy from the superior orbital foramen or the foramen magnum (Cook, 1996).

2.7 Treatment

Antimalarial agents may be used for the following purposes:

1. Treatment of a malarial attack
2. Suppression of an attack in the face of infection
3. Prophylaxis, and
4. Interference with transmission (Beaver & Jung, 1985).

However, the main aim in the treatment of a malarial attack or *Plasmodium* infection is to provide as rapid and certain relief as possible from the miseries and perils that accompany the erythrocytic infection (Beaver & Jung, 1985; Levinson & Jawetz, 1995).

Chloroquine and amodiaquine has been the mainstay of treatment until recent times when Africa has become confronted with drug resistance throughout most of the continent (Cook, 1996; Levinson & Jawetz, 1995). Several other drugs are currently in use including sulphadoxine pyrimethamine, artesunate and its derivatives, quinine, mepacrine, primaquine, halofantrine, mefloquine, proguanil, and in recent times, malarone® which is proguanil plus avotaquine, lapdap® which is chloroproguanil with dapsone and coartem®, which is artemether plus mefloquine. However, quinine still remains the drug of choice in the treatment of severe and/or chloroquine resistant malaria in most tropical countries (Hanscheid *et al.*, 2000). Prompt and effective treatment of malaria is one of the main strategies to reduce the intolerable burden of the disease in Africa, Ghana (WHO, 2006). This strategy reduces the evolution towards severe malaria and death. Due to the increasing resistance and failure of single drug treatments, WHO has since 2001, recommended that malaria endemic countries change their treatment policies and adopt combination therapy, and in particular artemisinin – based combination therapies (ACTs) (WHO, 2006). Based on evidence, the global consensus is currently in favour of ACTs as the first choice for the treatment of malaria when drug resistance to monotherapy is prevalent (WHO, 2006).

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The following ACTs are recommended for treatment of uncomplicated *P. falciparum* malaria in Africa on the basis of available efficacy and safety data:

- Artemether + lumefantrine (AM+LM)
- Artesunate + Amodiaquine (AS + AQ)

- Artesunate + Mefloquine (AS + MQ)
- Artesunate + sulfadoxine-pyrimethamine (AS + SP) (WHO, 2010a).

A national multi-sectoral task force formed in 2002 in response to the high and ever increasing levels of chloroquine resistance which approximated 23.2% in Ghana, reached a consensus with stakeholders to change the first line drug policy in 2004 (WHO, 2006). The consensus meeting selected AS + AQ as the preferred option for Ghana, based on local data on efficacy (WHO, 2006).

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WHO has also provided a guideline for the treatment of severe malaria (WHO, 2010a). Severe malaria is a medical emergency and after rapid clinical assessment and confirmation of the diagnosis, full doses of parenteral antimalarial treatment should be started without delay with whichever effective antimalarial is first available (WHO, 2010a). For adults, artesunate IV (intravenous) or IM (intramuscular): quinine is an acceptable alternative if parenteral artesunate is not available (WHO, 2010a).

The treatment of uncomplicated P. falciparum malaria in pregnancy provided by WHO is as follow:

First trimester:

- Quinine plus clindamycin to be given for 7 days (artesunate plus clindamycin for 7 days as indicated if this treatment fails)
- An ACT is indicated only if this is the only treatment immediately available, or if treatment with 7-day quinine plus clindamycin fails or uncertainty of compliance with 7-day treatment (WHO, 2010a).

Second and third trimesters:

- ACTs known to be effective in the country/region or artesunate plus clindamycin to be given for 7 days, or quinine plus clindamycin to be given for 7 days (WHO, 2010a).

Some antibacterial agents are known to have antimalaial activity, such as the sulphonamides and sulphones, which inhibit plasmodial folate synthesis by competing for the enzyme dihydropteroate synthetase (Cook, 1996).

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2.8 Prevention and control

Prevention and control of malaria have had their main objective as reduction of *Anopheles* below the transmission level since there is no effective vaccine as yet. As complementary line of action, transmission of plasmodia from man to mosquito could be prevented by treating infected people, providing chemoprophylaxis, and protecting infected as well as uninfected populations from anopheline vectors (Beaver & Jung, 1985).

This is embodied in the measures below:

1. Protection of human population from exposure to bites of *Anopheles*. This is provided through individual precaution such as:
    - Covering the exposed skin in the evening since the anopheline vectors are night biters.
    - Use of insecticide repellent creams containing dimethylphhthalate, dibutylphthalate or diethyltocamide.

- The use of efficient mosquito netting over the bed preferably impregnated with a synthetic pyrethroid such as permethrin or deltamethrin (Bell, 1990; Cahill & O'Brien, 1990).

2. Public health services could assist communities in the development of measures directed at destroying arthropod vectors such as mass spraying, or ensuring that every household has adequate mosquito-proof netting, or carrying out programmes to prevent breeding of *Anopheles* mosquitoes, such as larviciding and draining breeding sites for the mosquito vector. This breaks the life cycle of the parasite and thus reduces the hazards of individual and group exposure (Bell, 1990) .
3. Treatment of human infections with antimalarial drugs wherever practical. At times mass chemotherapy may be effective in preventing insects from acquiring and transmitting the parasite in endemic areas (Cahill & O'Brien, 1990).
4. Provision of chemoprophylaxis to people travelling to endemic areas.
5. Establishment of malaria surveillance programmes.
6. Institution of programmes to disperse practical advice to the public on preventive measures (Beaver & Jung, 1985; Levinson & Jawetz, 1995).

The number of malaria cases worldwide seems to be increasing, due to increasing transmission risk in areas where malaria control has declined, the increasing prevalence of drug resistant strains of parasites, and in a relatively few cases, massive increases in international travel and migration (Pasvol, 2005).

The World Health Organization (WHO) currently recommends a package of interventions for controlling malaria during pregnancy in areas with stable (high) transmission of *P. falciparum*, which includes the use of insecticide treated nets (ITNs), intermittent preventive treatment (IPT) with sulphadoxine-pyrimethamine (SP) and effective case management of malaria and anaemia (WHO, 2004). In order to reduce the burden of malaria in these women and its impact on anaemia, it may be essential to establish a system of supervised intermittent presumptive treatment with a safe and effective antimalarial so as to eliminate any parasites they may harbor (Shulman *et al.*, 1999). This will also help eliminate any asymptomatic parasitaemia capable of causing bone marrow suppression as has been reported (Shulman *et al.*, 1996). In southern Ghana, malaria in pregnancy and related morbidity are frequent (Mockenhaupt *et al.*, 2000a; Mockenhaupt *et al.*, 2006). The implementation of IPTp (Intermittent preventive treatment in pregnancy) was started in Ghana with three recommended doses of SP (Sulphadoxine pyrimethamine) at the end of 2004. In Ghana, SP achieves cure rates within 28 days of follow-up of 14% and 11% in children and pregnant women with uncomplicated malaria, respectively (Mockenhaupt *et al.*, 2005; Tagbor *et al.*, 2006). Further measures of malarial control, however, are needed to cover the vulnerable period of early pregnancy and the introduction of affordable insecticide treated nets (ITNs) are a suitable and effective option (ter Kuile *et al.*, 2003). Intermittent preventive therapy (IPT) with sulfadoxine-pyrimethamine (SP) has been shown to be superior to chemoprophylaxis or case management in the prevention of malaria in pregnancy and the use of ITN, chemophrophylaxis or case management in non-pregnant women of child-bearing age (Kayentao *et al.*, 2005).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted at the University Hospital, KNUST, Kumasi. Kumasi is the second largest city in Ghana, located in the rainforest zone of West Africa with a population of 1.5 million inhabitants (GSS, 2002). The climatic conditions of Kumasi are typical of that of a tropical region and therefore aid malaria transmission.

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The communities that surround KNUST which usually patronize the services of the University Hospital include; Ayigya, Bomso, Susuanso, Anloga, Oforikrom, Ahinsan, Atonsu, Ayeduase, Kotei, Kentinkrono, Boadi, Oduom, Anwomaso, Fumesua, Kwamo, and workers of the University. These communities have an average population of 12,601 inhabitants, with 8,200 being women (GSS, 2002). An average of 1800 pregnant women visit ANC every year, with about 150 visiting every month (KNUST, 2006).

3.2 Ethical consideration

Ethical clearance for the study was obtained from the Ethical Review Committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi. Permission to undertake the study at the University Hospital, KNUST, was sought and granted by the hospital management and the head of the laboratory. The subjects under study were provided with informed consent forms before they were recruited into the study.

3.3 Study design

The study was carried out between February, 2010 and December, 2010 at the University Hospital. A total of 760 subjects; made up of 380 pregnant women and 380 non-pregnant women of child-bearing age, who were attending the hospital, were recruited for the study. The study sample size was determined by using Epi Info version 6 statistical software. About 1.5 million people live in Kumasi of which 51% are women (GSS, 2002). Using a population size of 773,670 with expected frequency 45.0%, worst acceptable result of 50.0% and confidence interval of 95%, least study sample size of 380 was calculated.

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During their visits, after formal documented consent was obtained, demographic information such as age, parity, gestational period and preventive measures such as use of insecticide treated net (ITN), intermittent preventive treatment (IPT), use of insecticide sprays, mosquito coils, mosquito repellents and creams were recorded through a questionnaire. The insecticide sprays, mosquito coils, repellents and creams were classified as 'Others'.

Finger pricking was used for malaria detection, species identification using RDT and for measuring haemoglobin level.

3.4 Study materials

Materials including reagents and equipment used for the study have been outlined in Appendix 2.

3.5. Blood sample collection

3.5.1 Venipuncture

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The site for venipuncture was selected at the anticubital fossa area of the arm where the median cubital, cephalic, and basilic veins lie fairly close to the surface. With the tourniquet in place, the tip of the index finger was used to palpate (examine by feel) the vein. A vein was selected that was easily palpated, large enough to support good blood flow, and well- anchored by surrounding tissue. The venipuncture site was sterilized with 70% ethanol to help prevent infection and contamination of the specimen. The site was allowed to air dry (30 - 60 seconds) (NCCLS, 2000). Using a smooth motion, the needle was quickly inserted at an angle of 15-30 degrees and 1cm deep. The tourniquet was released as soon as blood (2 ml) was taken. After the required volume of blood had been taken, the patient was asked to release the fist; a clean gauze pad was placed firmly on the needle entry site. The needle was then gently and quickly removed from the arm. Each blood sample was released into a separate EDTA tube and labeled with an indelible marker or ink pen. The tube was inverted gently several times to mix the blood and anti-coagulant properly. The needles were disposed off in a biohazard container and the sample tubes delivered to the work bench within one hour for examination (NCCLS, 2000).

Figure 4. Collection of venous blood by venipuncture from non-pregnant woman

3.5.2 Finger pricking

Finger pricking was done to obtain blood sample to prepare smears for examination of malaria parasites. The third finger was selected holding the patient's left hand, palm upwards. The finger was cleaned with a piece of cotton wool lightly soaked in 70% ethanol, using firm strokes to remove dirt and grease from the ball of the finger. The finger was dried with a clean cotton wool, using firm strokes to stimulate blood circulation. The ball of the finger was punctured with a sterile lancet, using a quick rolling action. Gentle pressure was applied to the finger to express the first drop of blood and wiped away with a dry piece of cotton wool. Handling clean slides only by the edges, gentle pressure was applied to the finger and a single small drop of blood was collected for thin and thick film preparation. The slides were labeled with an indelible marker and delivered to the work bench (NCCLS, 2000).

Figure 5. Collection of blood by finger pricking from a pregnant woman

3.6 Laboratory processing of blood samples

3.6.1 Preparation of blood films

Thin and thick blood films were prepared. Absolute methanol was used to fix only the thin films. Thin blood films were used for species differentiation (confirm *Plasmodium* species) if not clear from thick films. The thin films were also examined to investigate anaemia (NCCLS, 2000).

3.6.1.1 Thin blood film

Thin blood film was prepared after finger pricking as described at section 3.5.2 above. A single small drop of blood was collected on the middle of the slide. Further pressure was applied to express more blood and two or three larger drops were collected on the slide, about 1 cm from the drop on the middle (NCCLS, 2000).

The remaining blood was wiped away from the finger with a piece of cotton wool. Using a second clean slide as a "spreader" and, with the slide with the blood drops resting on a flat, firm surface, the small drop was touched with the spreader and the blood allowed to run along its edge. The spreader was firmly pushed along the slide, keeping the spreader at an angle of 45°. The spreader was in even contact with the surface of the slide all the time the blood was being spread. The dried thin film was labeled with a soft lead pencil by writing across the thicker portion of the film, the patient's name or number and the date (NCCLS, 2000).

Figure 6: Holding spreader and slide with drops of blood, thin film

3.6.1.2 Thick blood film

Thick film was prepared after finger pricking (section 3.5.2). Slides were handled by the edges or by a corner to make thick film. Using the corner of the spreader, the remaining drops of blood after thin film were quickly joined and spread to make an even, thick film.

The blood was not excessively stirred but was spread in circular or rectangular form with 3 to 6 movements. The circular thick film was about 1 cm in diameter (NCCLS, 2000). The thick film was allowed to dry with the slide in a flat, leveled position, protected from flies, dust and extreme heat. The dried slides were dispatched to the work bench within one hour for examination after labeling (NCCLS, 2000).

Figure 7: Labeled thin blood film (A) and thick blood film (B)

3.6.2 Parasitological examination

The diagnosis of malaria was done by:

1. Detecting and identifying malaria parasites microscopically in blood film.
2. Using malaria rapid diagnostic test (RDT) to detect species of malaria parasite.

3.6.2.1 Malaria parasite detection by Giemsa staining

Malaria parasites were differentiated from platelets, RBCs, and WBCs using Giemsa stain containing azure B. The stain was diluted with buffered water to pH of 7.1-7.2. The aqueous working dilution of the stain (10% stain solution) was tested for proper staining reaction before being used at most for a day. Fresh whole blood was collected by finger pricking.

Thin and thick blood film slides were prepared using the fresh whole blood sample. The thin blood film was fixed in absolute methanol and allowed to dry whilst a thick blood film that has been allowed to dry thoroughly was not fixed. The test procedures were carried out in accordance with standard protocols (Appendix 3) (NCCLS, 2000).

Figure 8: Giemsa stained slides for microscopy

Figure 9: Positive slide showing ++++ of *P. falciparum*

3.6.2.2 Rapid diagnostic test (RDT)

Paracheck Pf cassette (Orchid Biomedical Systems, Verna, India) was used to detect *P. falciparum* HRP 2 antigen (Histidine-rich protein) in whole blood. The kit contains a swab, lancet, loop, and buffer. The second finger was cleaned using the swab. The finger was pricked with the lancet provided in the pouch. The loop was used to collect blood sample, later blotted onto sample pad A. Two drops of buffer was added to the buffer port B and the results read within 15 minutes. Positive test shows pink lines in both the control (C) band and test (T) band. Negative test shows pink line only in the control (C) band. The procedure for performing RDT is illustrated in appendix 4 (NCCLS, 2000).

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Figure 10: Test results of cassette malaria RDT

3.6.3 Haematological examination

Haematology analyzer (Medtrue Enterprise Co. Ltd., Jiangsu, China) was used to measure haemoglobin level in the pregnant and non-pregnant women to assess anaemia.

About 2 ml each of blood was obtained from the women and separately kept in an ethylene diamine tetra acetic acid (EDTA) containing tubes under aseptic condition. These tubes were placed under the analyzer to estimate the concentration of the haemoglobin in the various samples. Haemoglobin levels < 11 g/dl were reported as anaemic whilst those ≥ 11 g/dl were reported as non anaemic.

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3.7 Stool sample collection

Stool samples were collected from pregnant women to determine the presence of parasites. Consented subjects were provided with clean, dry, leak-proof, and wide-mouthed plastic specimen containers. They were given instructions on how to avoid contamination of stool sample with urine. The samples were labeled appropriately while those delivered to the laboratory later than two hours after collection or those less than 10 g were not included in the study. This was done in order to identify significant infective forms of the parasites. Each specimen was labeled with a study number, date and time of collection, and time the specimen was received (NCCLS, 2000).

3.8 Laboratory processing of stool sample

3.8.1 Stool macroscopy

Each specimen was first examined macroscopically and its consistency or state was recorded as either formed (F), semi-formed (SF), semi-formed with blood (SB), bloody mucoid (BM), loose (L) or watery (W). Samples were analyzed fresh, in batches, as soon as they were received; none was preserved in the refrigerator (NCCLS, 2000).

3.8.2 Stool microscopy

3.8.2.1 Direct wet mount

Fecal specimens were directly examined microscopically to detect parasite ova and larvae. Two-3 drops of fresh physiological saline was placed on a clean slide. The sample was emulsified very well to distribute evenly any parasites in the specimen. Using a wooden applicator stick, a small amount of the specimen about 2mg was mixed with the saline to make smooth thin preparation. Each preparation was covered with a cover slip. The entire field was systematically examined for larvae and nematode eggs. The ×10 objective was used with the condenser iris closed sufficiently to give good contrast. For the confirmation of eggs and larvae, ×40 objective was used. The test procedures were carried out in accordance with standard protocols (Appendix 5) (NCCLS, 2000). More sensitive concentration techniques were not used since it is a hospital based study (clinical cases), not an epidemiological investigation in a community.

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3.9 Quality control (QC)

3.9.1 Microscopy

1. The Binocular microscope (Beam Engineers, Haryana, India) which was used for this research was calibrated, and the objectives and oculars used for the calibration procedure were used for all measurements done with the microscope. The calibration factors (×10 and ×40 objectives) were posted on the bench beside the microscope for easy access.

2. Bench aids supplied by WHO for diagnosis of malaria parasites was used to ensure accurate identification of parasite species.
3. The reagents were checked for contamination each time they were used.
4. Specialized microscopists were employed to review the positive slides for confirmation (Garcia, 2001; NCCLS, 2000).

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3.9.2 Staining procedure

1. Visually, the thick smears were round to oval and approximately 2.0 cm across.
2. Visually, the thin films were rounded, feathered, and progressively thinner toward the middle of the slides.
3. The films did not have clear areas or smudges (indicating the absence of grease or fingerprints on the glass).
4. The stock buffer solutions and buffered water were clear, with no visible contamination.
5. Giemsa stain reagents were properly checked including the pH of the buffered water, before each use (Garcia, 2001; NCCLS, 2000).

3.10 Statistical analysis

Data were analysed using Microsoft Office Excel 2007, Epi Info version 6 and GraphPad Prism version 5.02 statistical softwares. Data were also presented as simple frequencies and percentages.

The chi square test was used in assessing the significance of associations between variables. Differences in proportions were analysed using the chi square or Fisher's exact test, if appropriate.

Univariate analyses were performed to determine which factors were significantly associated with malaria and anaemia, using Pearson Chi square tests for categorical variables and Student's t-tests for continuous variables. Multivariate analysis with parasitaemia (present/absent) or haemoglobin (anaemic/not-anaemic) categorized as a binomial outcome variable was performed using a mixed generalized linear model (GraphPad Prism). Odds ratios (OR) and coefficients were based on the final model only and include significant variables unless stated otherwise.

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The following factors were evaluated as potential risk factors for malaria parasitaemia and anaemia: age, gravidity, parity, intestinal helminthes, possession of ITN, use of IPT, and other preventive measures such as use of insecticide sprays, mosquito coils, mosquito repellents and creams.

3.10.1 Odds ratio (OR)

Odds ratios were calculated with 95% confidence interval (CI) to measure the strengths of the associations between variables. The odds ratio is one of a range of statistics used to assess the risk of a particular outcome (or disease) if a certain factor (or exposure) is present. The odds ratio is a relative measure of risk, indicating how much more likely it is that someone who is exposed to the factor under study will develop the outcome as compared to someone who is not exposed.

The odds of an event happening is the probability that the event will happen divided by the probability that the event will not happen. Odds ratio was referenced at 1.0.

If the odds are greater than one, then the event is more likely to happen than not. If the odds are less than one, then the event is less likely to happen than not. An odds ratio was used to compare the odds for the two groups.

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3.10.2 P-value

P-values provide a sense of the strength of the evidence against the null hypothesis. The smaller the P-value, the more strongly the test rejects the null hypothesis, that is, the hypothesis being tested. The most commonly used level of significance is 0.05. For all statistical tests, two-sided P-value < 0.05 was considered significant.

Therefore, the null hypothesis will be rejected in favour of the alternative hypothesis. Also, if the P-value is less than 0.05, then the 95% confidence interval cannot contain the value that defines the null hypothesis.

CHAPTER FOUR

4.0 RESULTS

A total of 380 pregnant and 380 non-pregnant women of child-bearing age were enrolled into the study between February 2010 and December 2010.

4.1 Demographic characteristics of studied population

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Demographic features of the population studied include: age, parity, gravidity and gestational age.

4.1.1 Age distribution

The age of the pregnant women and non-pregnant women of child-bearing age ranged between 16-45 years. Women were categorized into "young" or "old" if they were less than 20 years or 20 years and above respectively. Ten (2.6%) pregnant and 29 (7.6%) non-pregnant women of child-bearing age were considered young. Three hundred and seventy (97.4%) of the pregnant women whilst 351 (92.4%) non-pregnant women of child-bearing age respectively were also considered old (Tables 1 and 2).

Table 1: Prevalence of malaria parasitaemia in pregnant women

	n (%)	Prevalence n (%)	Odds Ratio (95% CI)	P-value
Overall (Subjects)	380 (100)	48 (12.6)		
Maternal Age				
< 20 years	10 (2.6)	5 (50.0)	7.61 (2.114- 27.35)	0.0041
≥ 20 years*	370 (97.4)	43 (11.6)		
Parity				
Nulliparous	113 (29.7)	22 (19.5)	2.24 (1.209-4.153)	0.0114
≥ 1 Births*	267 (70.3)	26 (9.7)		
Gravidity				
Primigravidae	100 (26.3)	14 (14.0)	1.18 (0.6032-2.300)	0.6037
Multigravidae*	280 (73.7)	34 (12.1)		
Trimester				
First-trimester	111 (29.2)	10 (9.0)	0.25 (0.09735-0.6293)	0.0039
Second-trimester	227 (59.7)	26 (11.5)	0.32 (0.1476-0.7086)	0.0068
Third-trimester*	42 (11.1)	12 (28.6)		
Preventive Methods				
ITN				
Yes	251 (66.1)	17 (6.8)	0.23 (0.1215-0.4342)	< 0.0001
No*	129 (33.9)	31 (24.0)		
IPT				
Yes	0 (0.0)	0 (0.0	-	-
No*				
Others				
Yes	221 (58.2)	40 (18.1)	4.17 (1.894-9.186)	0.0001
No*	159 (41.8)	8 (5.0)		

* = Reference Category; CI= Confidence interval; n = Number of subjects

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Table 2: Prevalence of malaria parasitaemia in non-pregnant women of child-bearing age

	n (%)	Prevalence n (%)	Odds Ratio (95% CI)	P-value
Overall (Subjects)	380 (100)	25 (6.6)		
Age				
< 20 years	29 (7.6)	3 (10.3)	1.73 (0.4841-6.150)	0.4239
≥ 20years*	351 (92.4)	22 (6.3)		
Preventive Method				
ITN				
Yes	273 (71.8)	13 (4.8)	0.40 (0.1745-0.8981)	0.0358
No*	107 (28.2)	12 (11.2)		
Prophylaxis				
Yes	95 (25.0)	3 (3.2)	0.39 (0.1140-1.333)	0.1533
No*	285 (75.0)	22 (7.7)		
Others				
Yes	232 (61.1)	8 (3.4)	0.28 (0.1156-0.6554)	0.0027
No*	148 (38.9)	17 (11.5)		

* = Reference Category; CI= Confidence interval; n = Number of subjects.

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4.1.1.1 Age and malaria parasitaemia

There was 50% and 10.3% malaria prevalence in both young pregnant and young non-pregnant women respectively. This gave an Odds Ratio (OR) of 7.61 at 95% CI (2.1-27.4) in the pregnant women and an OR of 1.73 at 95% CI (0.5-6.2) in the non-pregnant women of child-bearing age. Among the old women, 43 (11.6%) of pregnant women and 22 (6.3%) of the non-pregnant women of child-bearing age had malaria parasites (Tables 1 and 2).

4.1.1.2 Age and anaemia

Anaemia was rated based on the haemoglobin level of the subjects. The prevalence of anaemia at older age was higher than at younger age. Among women aged < 20 years, 6 (60.0%) pregnant women and 17 (58.6%) non-pregnant women of child-bearing age had anaemia (Tables 3 and 4). At age < 20 years, there was a higher risk of anaemia (OR = 1.27, 95% CI = 0.6-2.7) among the non-pregnant women of child-bearing age than among the pregnant women (OR = 0.90, 95% CI = 0.2-3.2). The prevalence of anaemia in pregnant women was higher than in non-pregnant women of child-bearing age at age ≥ 20 years. At age ≥ 20 years, 232 (62.7%) of pregnant women and 185 (52.7%) of the non-pregnant women were anaemic (Tables 3 and 4).

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Table 3: Anaemia in pregnant women

	n (%)	Prevalence Hb<11 g/dl n (%)	Odds Ratio Hb<11 g/dl (95% CI)	Prevalence Hb≥11 g/dl n (%)	Odds Ratio Hb≥11 g/dl (95% CI)	P-value
Overall (Subjects)	380 (100)	238 (62.6)		142 (37.4)		
Maternal Age						
< 20 years	10 (2.6)	6 (60.0)	0.90 (0.2474-3.218)	4 (40.0)	1.12 (0.3107-4.043)	1.0000
≥ 20 years*	370 (97.4)	232 (62.7)		138 (37.3)		
Parity						
Nulliparous	113 (29.7)	68 (60.2)	0.86 (0.5488-1.355)	45 (39.8)	1.16 (0.7382-1.822)	0.5624
≥1 Births*	267 (70.3)	170 (63.7)		97 (36.3)		
Gravidity						
Primigravidae	100 (26.3)	50 (50.0)	0.49 (0.3075-0.7788)	50 (50.0)	2.04 (1.284.-3.252)	0.0027
Multigravidae*	280 (73.7)	188 (67.1)		92 (32.9)		
Trimester						
First-trimester	111 (29.2)	71 (64.0)	0.80 (0.3719-1.702)	40 (36.0)	1.26 (0.5874-2.689)	0.7037
Second-trimester	227 (59.7)	138 (60.8)	0.70 (0.3429-1.409)	89 (39.2)	1.44 (0.7098-2.916)	0.3874
Third-trimester*	42 (11.1)	29 (69.0)		13 (31.0)		
Preventive Methods						
ITN						
Yes	251 (66.1)	148 (58.9)	0.62 (0.3961-0.9787)	103 (41.1)	1.61 (1.022-2.524)	0.0440
No*	129 (33.9)	90 (70.4)		39 (29.6)		
IPT						
Yes	0 (0.0)	0 (0.0)	-	-	-	-
No*						
Others						
Yes	221 (58.2)	136 (61.5)	0.89 (0.5860-1.364)	85 (38.1)	1.12 (0.7330-1.707)	0.6674
No*	159 (41.8)	102 (64.2)		57 (36.0)		
Stool						
Nematodes	5 (1.3)	2 (40.0)	0.39 (0.06478-2.380)	3 (60.0)	2.55 (0.4202-15.44)	0.3668
No Nematode*	375 (98.7)	236 (62.9)		139 (37.1)		
Malaria Parasites						
Postitive	48 (12.6)	24 (50.0)	0.55 (0.2999-1.014)	24 (50.0)	1.81 (0.9864-3.334)	0.0571
Negative*	332 (87.4)	214 (64.5)		118 (35.5)		

* = Reference Category; CI= Confidence interval; n = Number of subjects; Hb = Haemoglobin level

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Table 4: Anaemia in non-pregnant women of child-bearing age

	n (%)	Prevalence Hb<11 g/dl n (%)	Odds Ratio Hb<11 g/dl (95% CI)	Prevalence Hb≥11 g/dl n (%)	Odds Ratio Hb≥11 g/dl (95% CI)	P-value
Overall (Subjects)	380 (100)	202 (53.2)		178 (46.8)		
Age						
< 20 years	29 (7.6)	17 (58.6)	1.27 (0.5896-2.741)	12 (41.4)	0.79 (0.3649-1.696)	0.5676
≥ 20 years*	351 (92.4)	185 (52.7)		166 (47.3)		
ITN						
Yes	273 (71.8)	138 (50.5)	0.69 (0.4364-1.081)	135 (49.5)	1.46 (0.9251-2.292)	0.1107
No*	107 (28.2)	64 (59.8)		43 (40.2)		
Others						
Yes	232 (61.1)	127 (54.7)	1.18 (0.7788-1.780)	105 (45.3)	0.85 (0.5619-1.284)	0.4617
No*	148 (38.9)	75 (50.7)		73 (49.5)		
Malaria Parasites						
Positive	25 (6.6)	19 (76.0)	2.98 (1.161-7.630)	6 (24.0)	0.34 (0.1311-0.8613)	0.0217
Negative*	355 (93.4)	183 (51.5)		172 (48.5)		

* = Reference Category; CI= Confidence interval; n = Number of subjects; Hb = Haemoglobin level.

4.1.2 Parity

One hundred and thirteen (29.7%) of the pregnant women were nulliparous whilst 267 (70.3%) were multiparous. Malaria parasitaemia was higher in nulliparous women than multiparous women. Malaria parasitaemia was found in 22 (19.5%) nulliparous women and 26 (9.7%) women who had one or more pregnancies with successful delivery. Compared to multiparous women, there was a significantly higher risk of malaria parasitaemia in the nulliparous women (OR=2.24) (Table 1). Sixty eight (60.2%) of the nulliparous women and 170 (63.7%) multiparous women were anaemic. There was lower risk of anaemia in the nulliparous women (OR=0.86) using multiparity as reference category (Table 3).

4.1.3 Gravidity

The women studied were divided into two gravidae; primigravidae and multigravidae. The primigravidae were in their first pregnancy whilst multigravidae had had two or more pregnancies. One hundred (26.3%) of the pregnant women were primigravidae whilst 280 (73.7%) were multigravidae (Table 1). The number of primigravidae and multigravidae with malaria parasites in their blood samples were 14 and 34 respectively. The prevalence of malaria parasitaemia in multigravidae (12.1%) was lower than primigravidae (14.0%). There was slightly higher risk among the primigravidae (OR = 1.18, 95% CI=0.6-2.3) using multigravidae as a reference category (Table 1).

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The prevalence of anaemia was higher in the multigravidae than in the primigravidae. About 50.0% primigravidae and 67.1% multigravidae were anaemic with lower risk in the primigravidae (OR = 0.86, 95% CI = 0.5-1.4) (Table 3).

4.1.4 Gestational period

The gestational period was divided into three stages; first trimester (0-12 weeks), second trimester (13-24 weeks) and third trimester (25-40 weeks). In the pregnant women, 111 (29.2%), 227 (59.7%), and 42 (11.1%) were in the first trimester, second trimester and third trimester respectively. This shows that most of the pregnant women who attended the antenatal clinic were in their second trimester. The prevalence of malaria was 10 (9.0%) in the first trimester, 26 (11.5%) in the second trimester, and 12 (28.6%) in the third trimester.

Odds ratios of 0.25 at 95% CI (0.10-0.63), and 0.32 at 95% CI (0.15-0.71) were recorded in the first and second trimesters respectively with significant P-values (P-value = 0.0039 for first trimester, P-value = 0.0068 for second trimester) (Table 1).

The prevalence of anaemia was 71 (64.0%), 138 (60.8%), 29 (69.0%) in the first, second, and third trimesters respectively. Pregnant women in the third trimester recorded the highest prevalence of anaemia. The risk of anaemia was higher in the first trimester as compared to those in second trimester (OR=0.80 at 95% CI=0.38-1.70 versus OR=0.70 at 95% CI=0.34-1.41 for first and second trimesters respectively) (Table 3).

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4.2 Preventive measures

Preventive methods used by the pregnant women and non-pregnant women of child-bearing age included the use of Insecticide treated nets (ITN), Prophylaxis and 'others'. The 'others' comprised insecticide sprays, mosquito coils, mosquito repellents and creams. Two hundred and fifty one (66.1%) pregnant women used ITN, 0 (0.0%) used IPT, and 221 (58.2%) used 'Others', whilst 273 (71.8%) non-pregnant women of child-bearing age used ITN, 95 (25.0%) used prophylaxis, and 232 (61.1%) used 'others' (Tables 1 and 2). There was a significantly high level of ITN usage and 'others' as preventive measures in pregnant women ($P < 0.0001$ for ITN and $P = 0.0001$ for 'others') (Table 1).

The prevalence of malaria in pregnant women showed that, 17 (6.8%) were ITN users, 0 (0.0%) were IPT users, and 40 (18.1%) used 'Others'. There was a high risk in the use of 'Others' (OR = 4.17, 95% CI = 1.90-9.19, P-value = 0.0001) as a preventive measure but low risk was associated with the use of ITN (OR = 0.23, 95% CI = 0.12-0.43, P-value < 0.0001) (Table 1). Among the non-pregnant women of child-bearing age, malaria parasitaemia was found in 13 (4.8%) ITN users, 3 (3.2%) prophylaxis and 8 (3.4%) 'others' users (Table 2). There was low risk of malaria parasitaemia in the non-pregnant women of child-bearing age in relation to preventive measures; (OR = 0.40, 95% CI = 0.17-0.90 for ITN, OR = 0.39, 95% CI = 0.11-1.33 for prophylaxis, and OR = 0.28, 95% CI = 0.12-0.66 for 'others') (Table 2).

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In pregnant women, anaemia was recorded in 148 (58.9%) ITN users, 0 (0.0%) IPT users, and 136 (61.5%) 'others' users. Odds ratios of 0.62 at 95% CI (0.40-0.98) for ITN usage, and 0.89 at 95% CI (0.59-1.36) for 'others' was deduced from the analysis. This was an indication of low risk anaemia associated with preventive methods (Odds ratios < 1). Comparing this with non-pregnant women of child-bearing age revealed that, 138 (50.5%) ITN users, and 127 (54.7%) who used other means of control were anaemic (Tables 3 and 4). There was no significant association between anaemia and the use of 'others' (OR = 1.18, 95% CI = 0.78-1.78) whilst low risk was associated with the use of ITN (OR = 0.69, 95% CI = 0.44-1.08) in the non-pregnant women (Table 4). There was higher non-anaemic cases in 'others' (45.3%) as preventive measure in non-pregnant women compared to 41.1% pregnant women who used ITN (Tables 3 and 4).

4.3 Intestinal nematodes

Intestinal nematodes found in the stool specimens analysed included; *Ascaris lumbricoides* (2), *Trichuris trichiura* (1), and *Strongyloides stercoralis* (2). Forty percent of the pregnant women with intestinal nematodes had anaemia. Anaemia in pregnant women was not significantly associated with intestinal nematodes (OR = 0.39; 95% CI = 0.06-2.38, P-value > 0.05) (Table 3). Co-infection of malaria with intestinal nematodes was not recorded in the pregnant women.

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4.4 Species differentiation

The species of *Plasmodium* found in the pregnant women were *Plasmodium falciparum* (85.4%), *Plasmodium ovale* (10.4%) and *Plasmodium malariae* (4.2%). Among the non-pregnant women of child-bearing age, 76% P. *falciparum, 16% P. ovale,* and 8% *P. malariae* were also found (Table 5).

Table 5: *Plasmodium* species infecting pregnant and non-pregnant women of child-bearing age

Women	Species Present	Frequency	Percentage (%)
Pregnant	*P. falciparum*	41	85.4
	P. ovale	5	10.4
	P. malariae	2	4.2
Non-pregnant	*P. falciparum*	19	76
	P. ovale	4	16
	P. malariae	2	8

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4.5 Malaria in pregnant and non-pregnant women of child-bearing age

Relating the variables under study with malaria infection, malaria parasitaemia was higher in pregnant women compared to non-pregnant women of child-bearing age. This is illustrated in the Figure 11 below.

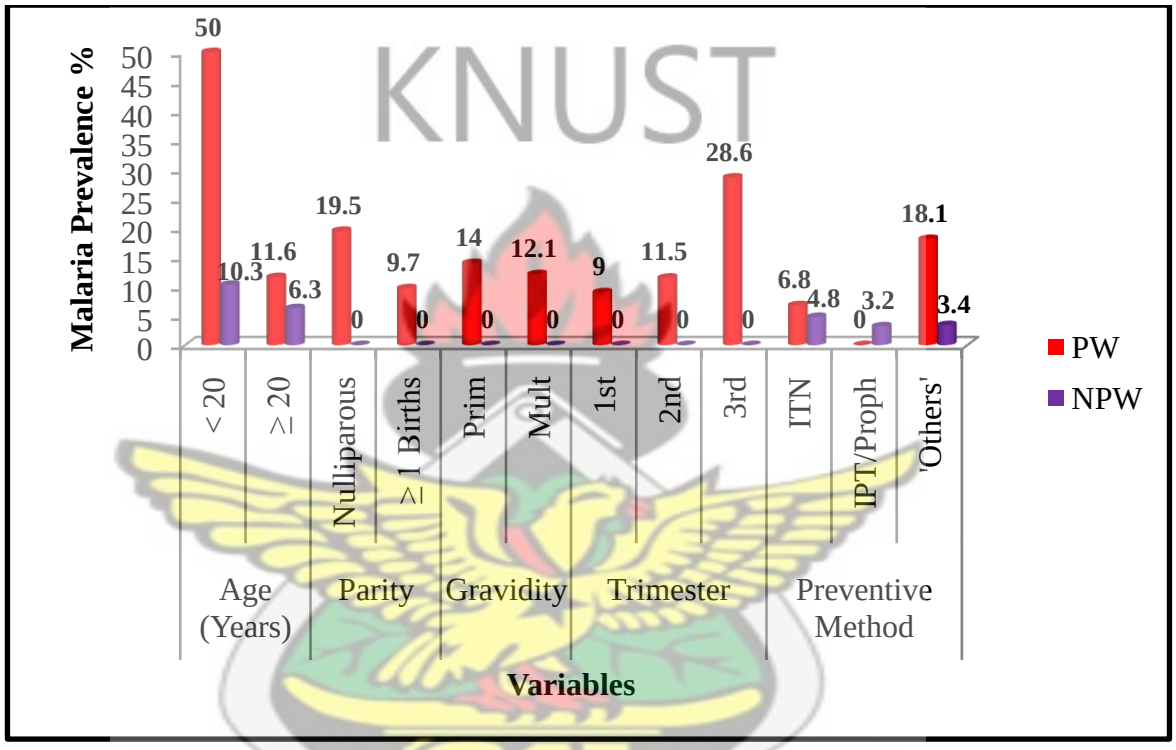

Figure 11: Comparison of malaria parasitaemia in pregnant and non-pregnant women of child-bearing age.

PW = Pregnant women, NPW = Non-pregnant women, Proph=Prophylaxis, 1st= First trimester, 2nd=Second trimester, 3rd=Third trimester, Prim=Primigravidae, Multi=Multigravidae.

4.6 Anaemia in pregnant and non-pregnant women of child-bearing age

Correlating the variables under study with anaemia, most of the variables showed high anaemia prevalence in pregnant women compared to non-pregnant women of child-bearing age. This is illustrated in the Figure 12 below.

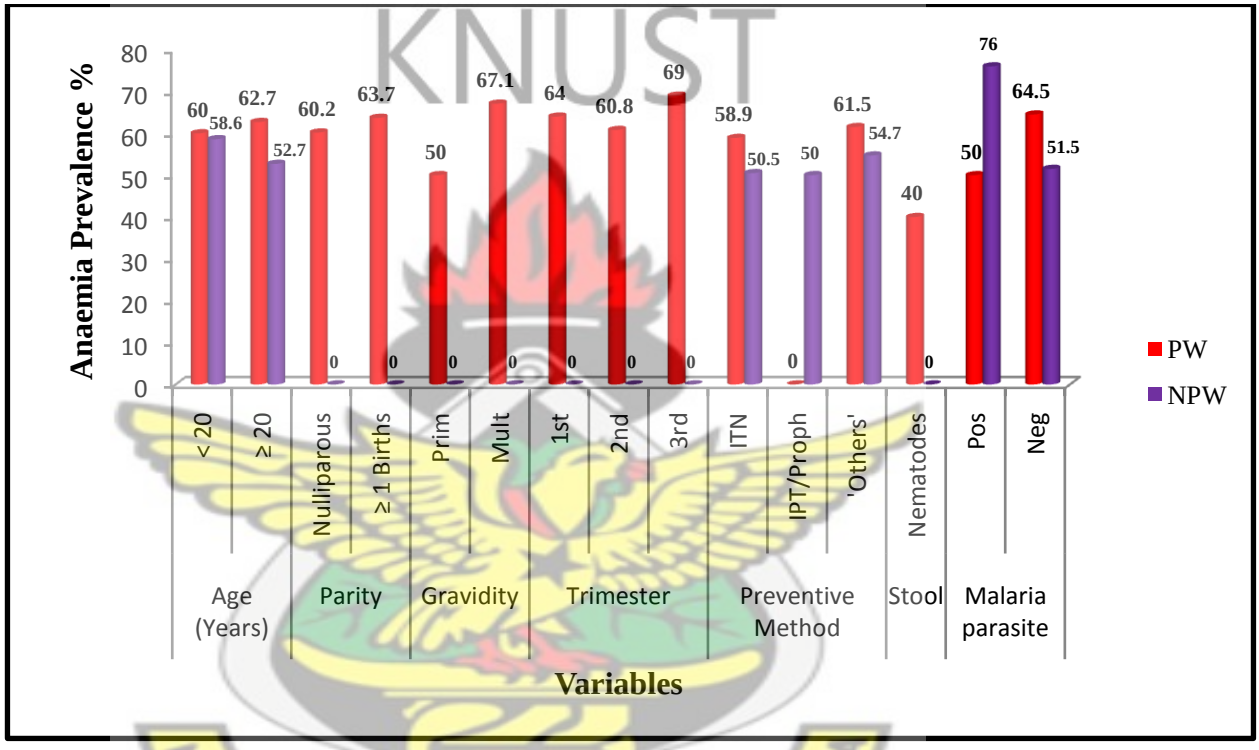

Figure 12: Comparison of anaemia in pregnant and non-pregnant women of child-bearing age.

PW = Pregnant women, NPW = Non-pregnant women, Proph=Prophylaxis, 1st= First trimester, 2nd=Second trimester, 3rd=Third trimester, Prim=Primigravidae, Multi=Multigravidae, Pos=Positive, Neg=Negative

4.7 Malaria and anaemia

In summary, prevalence of malaria and anaemia were higher in pregnant women compared with their non-pregnant counterparts. Pregnant women had 12.6% malaria prevalence whilst non-pregnant women of child-bearing age had 6.6%. Anaemia prevalence in pregnant and non-pregnant women were 62.6% and 53.2% respectively. This is shown in Figure 13 below.

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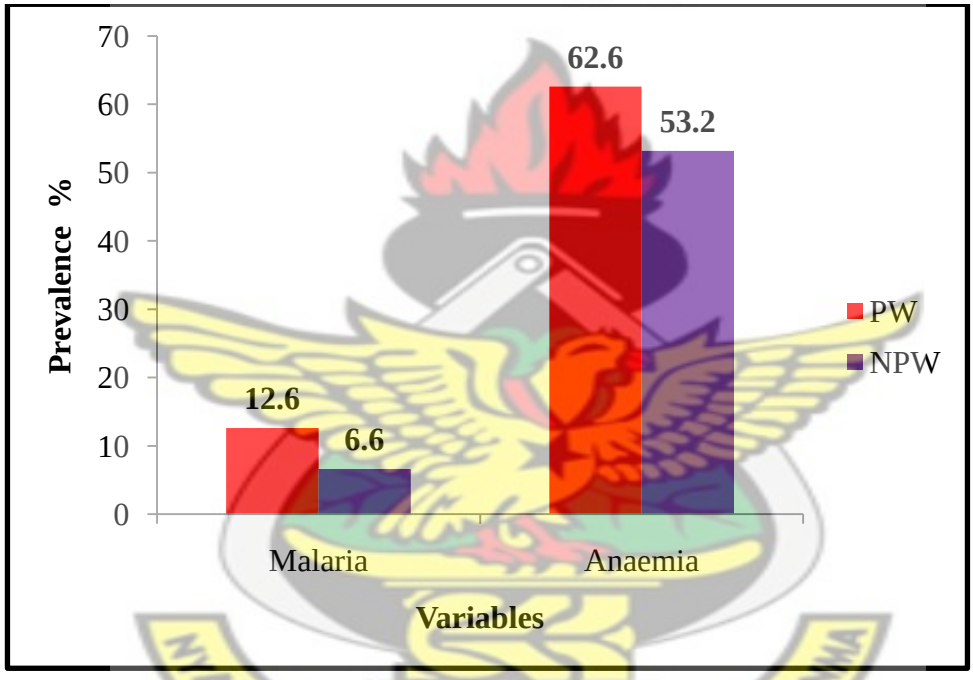

Figure 13: Comaprison of malaria and anaemia in pregnant and non-pregnant women of child-bearing age.

PW = Pregnant women, NPW = Non-pregnant women.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Demographic characteristics

5.1.1 Effect of age distribution

The age of pregnant and non-pregnant women of child-bearing age ranged from 16 to 45 years. Most of the respondents under study were old (≥ 20 years) suggesting low level of teenage pregnancy in the study communities. This might be due to education, creating awareness and also the fact that most of the women living in these areas were above 20 years. Malaria parasitaemia differed between age groups. At age ≥ 20 years, the prevalence of malaria was higher in pregnant women compared to non-pregnant women of child-bearing age. In general, parasite density was higher in the older than the younger women. This contradicts a study in Gabon (Bouyou-Akotet *et al.*, 2003) with younger women more at risk. This may be due to host or environmental factors as reported by Bouyou-Akotet *et al*. (2003). This may also be attributed to age related immunity as a result of previous exposure to malaria in child-bearing years (Bouyou-Akotet *et al.*, 2003).

Anaemia prevalence was higher at older ages (62.7%) in pregnant women but lower (52.7%) in the non-pregnant women of child-bearing age. This contradicts a study in Ghana where lower prevalence of anaemia was significantly associated ($r = 0.86$, $p < 0.001$) with older age of pregnant women (Glover-Amengor *et al.*, 2005).

5.1.2 Effect of parity

The incidence of malaria parasitaemia was higher in the nulliparous (19.5%) than the multiparous (9.7%) pregnant women. This is in agreement with other studies which indicated that with successive births, women are subsequently exposed to a variety of strains of malaria and develop efficient mechanisms to control infection leading to the prevention of malaria (Beeson *et al.*, 2000; Fried & Duffy, 1996). Shulman & Dorman (2003) showed that peripheral and placental parasitaemia decrease with increasing parity among pregnant women, a finding that supports the results of the present study. The present study revealed that, parity is a variable that affects malaria parasitaemia (P-value < 0.05).

Multiparous women had higher prevalence (67.1%) of anaemia than the nulliparous (50.0%). In a study in Iran, Veghari *et al.* (2007) found similar results. Glover-Amengor *et al.* (2005) in their study reported lower prevalence of anaemia which was strongly associated with increasing parity of pregnant women ($r = 0.7$, $p = 0.003$). Andert *et al.* (2006) reported no difference in prevalence of anaemia in the pair groups (Multiparous and nulliparous). Decrease of iron from tissue, malnutrition and insufficient iron consumption are contributory factors to the above problem (Veghari *et al.*, 2007). Therefore multiparity is a contributory factor to anaemia (Veghari *et al.*, 2007).

5.1.3 Effect of gravidity

Malaria was more prevalent in primigravidae (14.0%) compared to multigravidae (12.1%). This finding was not different from a study done in Gabon by Bouyou-Akotet *et al*. (2003). Shulman *et al.* (2002) reported the same finding where 64% primigravidae and 30% multigravidae had malaria. The report by Shulman *et al.* (2002) showed higher prevalence of malaria compared to the present study. Other studies also reported that primigravidae were more heavily and more often infected (up to twice as much) than multigravidae (Brabin, 1991; McGregor, 1984; Menendez, 1995). Anti-adhesion antibodies against chondroitin sulphate A-binding parasites are associated with protection from maternal malaria, but these antibodies develop only over successive pregnancies, hence the susceptibility of primigravidae to malaria infection compared to multigravidae (Duffy & Fried, 1999). Some authors also believed that primigravid women have little or no immunity against the infecting strains of *Plasmodium* and hence suffer adverse complications more common in primigravida than multigravida (Beck *et al.*, 2001; Nwonwu *et al.*, 2009). Occurrence of anaemia was relatively more in multigravidae (67.1%) than primigravidae (50.0%). This finding confirms earlier studies conducted in Nigeria where haemoglobin level < 11g/dl was found less in primigravidae (35.5%) (Nwonwu *et al.*, 2009). This may be due to repeated pregnancies at shorter intervals without allowing for replenishment of the iron stores (Nwonwu *et al.*, 2009).

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5.1.4 Effect of gestational age

Many of the respondents in this study registered for antenatal care in their second and third trimester. This seems to be a common practice in Africa (Steketee *et al.*, 1988). In a study conducted in Zaire, most of the pregnant women attended antenatal clinic for the first time in their sixth or seventh month of gestation and made three to four visits before delivery (Steketee *et al.*, 1988). This practice is detrimental as it does not allow for early detection and correction of pregnancy complications, such as anaemia. It is therefore very important to educate women on the need to register early for antenatal care. Among the respondents that had malaria, 9.0% were in their first trimester of pregnancy and 11.5% in their second trimester. This finding confirms results of similar study conducted in Nigeria (Okonofua & Abejide, 1996). It is known that in holo-endemic areas, parasite density and clinical malaria are most prevalent in the first trimester and early second trimester of pregnancy, as this corresponds to the period during which there is the most significant decrease in humoral and cell-mediated immunity to malaria (Nwonwu *et al.*, 2009). The mean Hb for the pregnant women in the last trimester in the present study was relatively low (9.4 g/dl) compared to an earlier study in Ghana with a mean Hb value of 10.7 g/dl (Mockenhaupt *et al.*, 2000b). Women in the third trimester had the highest anaemia prevalence (69.0%) compared to 60.8% in the second trimester and 64.0% during the first trimester. Statistically, this result was due to the lower number of pregnant women who attended ANC during their third trimester in the present study. In their study, Ouma *et al.* (2007) found that anaemia was strongly associated with pregnant women in the second trimester.

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It is possible that better nutrition or increased iron intake may have resulted in reduced anaemia, but we have no information on the diet of participants to evaluate this hypothesis.

5.2 Preventive measures

Malaria parasitaemia was found in 6.8% pregnant women and 4.8% non-pregnant women of child-bearing age who used ITN as preventive measure. The association between ITN use and malaria parasitaemia was statistically significant ($P < 0.05$). Similar study in Kenya showed 13.1% malaria parasitaemia prevalence with non-significant P- value (Ouma *et al.*, 2007). ITNs reduce human-vector contact by physically excluding vector mosquitoes, killing them if they land on ITNs or repelling them, thereby driving them away. Distribution of ITNs through ANC can help, but this does not address the effects of malaria before the first ANC visit (Guyatt *et al.*, 2002).

Anaemia was also found to be 58.9% in pregnant women and 50.5% in non-pregnant women of child-bearing age who used ITNs. This prevalence is low compared to a study in Tanzania where 72% pregnant ITN users were anaemic (Marchant *et al.*, 2002). With a significant P-value, ITN usage is effective in controlling anaemia during pregnancy. This is supported by other studies where ITNs effectively reduce the prevalence of anaemia during pregnancy and women should be encouraged to use them (Gamble *et al.*, 2006; ter Kuile *et al.*, 2003). Among non-pregnant women, a study in Nigeria showed lower anaemia prevalence (Erhabor *et al.*, 2010) compared with the present study.

The use of 'others' which comprised insecticide sprays, mosquito coils, mosquito repellents and creams made a significant impact on malaria parasitaemia in both pregnant and non-pregnant women of child-bearing age. In Uganda mosquito coils were rarely used in malaria prevention by pregnant women (Mbonye *et al.*, 2005). This is due to its possible health hazard, socioeconomic status, chest congestion, bad smell, duration and potency of the chemicals in the insecticide sprays, mosquito coils, mosquito repellents and creams (Mbonye *et al.*, 2005). Although recommended, none of the pregnant women in the present study received IPT at ANC. This contradicts and earlier study where 26.4% of the women did not receive an IPT during ANC and only 34.2% received two doses (Yatich *et al.*, 2009).

In order to reduce the burden of malaria in pregnant women and its impact on anaemia, it may be essential to establish a system of supervised intermittent preventive treatment with a safe and effective anti-malarial so as to control or reduce malaria parasitaemia in pregnant women (Shulman *et al.*, 1999).

5.3 Prevalence of intestinal nematodes

Five (1.3%) cases of intestinal nematodes were reported in the present study. This finding was low compared to an earlier study where an overall prevalence of 25.7% intestinal nematodes was observed in pregnant women (Yatich *et al.*, 2009). A study in Kenya contradicts the present study where 73% pregnant women had intestinal nematodes (Geissler *et al.*, 1999).

In the present study, two (40.0%) pregnant women with intestinal nematodes had anaemia with non-significant P-value. Moreover, Vasanthi *et al.* (1994) in their earlier studies in Kenya and Zanzibar reported significant association between anaemia and intestinal nematodes.

5.4 *Plasmodium* species infecting the study population

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Three species of *Plasmodium* (*P. falciparum, P. malariae, and P. ovale*) were identified in the study to cause malaria and the highest number isolated in pregnant women in their second trimester. The predominant species isolated was *P. falciparum* in both pregnant and non-pregnant women of child-bearing age. This is because, *P. falciparum* is the main species found in the tropical and subtropical Africa (Cheesbrough, 2005). *P. falciparum* is also the most widespread species, accounting for up to 80% of malaria cases worldwide (Cheesbrough, 2005). The present study agreed with a study in Pakistan (Saba *et al.*, 2008), indicating 76.75% *P. falciparum* infection during pregnancy. In India, 58% *P. falciparum* infection was also recorded in the non-pregnant women of child-bearing age (Singh *et al.*, 1995). *P. falciparum* prevalence and density were higher among pregnant women (85.4%) compared with non-pregnant women of child-bearing age (76%) in the present study. This might be due to depressed cellular immune responses to *P. falciparum* antigens in pregnant women in comparison with non-pregnant women of child-bearing age (Fievet *et al.*, 1995).

5.5 Prevalence of malaria in pregnant and non-pregnant women

The study showed prevalence of malaria parasitaemia to be 12.6% among pregnant women and 6.6% among non-pregnant women of child-bearing age. Nduka *et al.* (2006) also reported higher malaria parasitaemia in pregnant women (54%) compared with non-pregnant women of child-bearing age (33%). In the present study, 12.6% prevalence of malaria parasitaemia in pregnant women was low compared with 23% reported in Mozambique (Saute *et al.*, 2002) and 26.75% reported in Malawi (Rogerson *et al.*, 2003).

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The prevalence of malaria in the present study among pregnant women was much lower than 47.5 % reported in Onitsha (Nwokedi, 1992), 42% reported in Ghana (Mockenhaupt *et al.*, 2000b), 57.5% reported in Gabon (Bouyou-Akotet *et al.*, 2003) and 41% observed in Uyo, Nigeria (Opara *et al.*, 2004).

The high rate of malaria prevalence observed in the present study could be due to the environmental conditions inherent in urban and peri-urban areas, which favour malaria transmission (Nduka *et al.*, 2006). It has been recognized that a temperature range of 20^0C–30^oC and relative humidity of 60% and above were suitable for malaria parasite transmission (WHO, 2000). The attitude of the women of not starting pre-natal care early in pregnancy may also have contributed to this prevalence (Nduka *et al.*, 2006). Some of the women began pre - natal care either towards the end of their 1st trimester or mid second trimester (Nduka *et al.*, 2006).

Also, there is a transient depression of cell-mediated immunity in pregnancy that allows foetal allograft retention but also interferes with resistance to various infectious diseases such as malaria (Meeusen *et al.*, 2001).

5.6 Anaemia in pregnant and non-pregnant women of child-bearing age

Anaemia prevalence was higher in pregnant women (62.6%) compared to 53.2% in non-pregnant women of child-bearing age. Nduka *et al.* (2006) agreed with the higher anaemia prevalence in pregnant women (77%) compared with non-pregnant women of child-bearing age (29%). Similar result was found in other studies (Nestel *et al.*, 1999; Sadeghipour *et al.*, 2001; Stevens, 2000). The prevalence of anaemia in the present study was in agreement with mean percentage for Africa put at 61 % (Nduka *et al.*, 2006). It also agreed with studies of Nair and Nair (1993) in Tanzania, Van Den Broek *et al.* (2000) in Southern Malawi and Bouyou - Akotet *et al.* (2003) in Gabon.

5.7 Malaria and anaemia

Malaria and anaemia are important public health problems in the study population and pregnant women are the most vulnerable. Van den Broek (1996a) also reported pregnant women and children under five years as the most vulnerable. In the present study no significant association was found between intestinal nematodes and anaemia (P-value > 0.05). Moreover, malaria related anaemia of 50% (24 out of 48 subjects) was found to be less than the total anaemia prevalence of 62.6% (238 out of 380) in pregnancy.

Therefore, it is likely saying, 'malaria was not the only cause of anaemia in pregnant women' except in non-pregnant women of child-bearing age. This cannot be justified because of the less sensitive wet mount technique used in isolating intestinal helminthes. But then, Whitfield (1995) and Van den Broek (1996b) reported anaemia in pregnancy as multi-factorial and may be caused by other factors other than malaria parasitaemia. A study in Gabon also showed that pregnant women with low anaemia had the highest prevalence of malaria (50%), and higher parasite load was common in pregnant women with severe anaemia (Bouyou-Akotet *et al.*, 2003). Similar finding was reported in Balantyre, Malawi (Rogerson *et al.*, 2000). In the present study, age, gestational period and parity did not influence the prevalence of anaemia.

A successful strategy to combat malaria and anaemia, therefore, should address all the potential causal factors after their elucidation.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In the study population, malaria parasitaemia and anaemia were common medical conditions associated with pregnancy. Pregnant women were more susceptible to malaria parasitaemia and anaemia than non-pregnant women of child-bearing age. Malaria was not the only cause of anaemia and the main species of *Plasmodium* isolated in the blood samples was *Plasmodium falciparum*.

Factors associated with malaria parasitaemia include; maternal age, gestational period, nulliparity, ITN, and "others" as preventive method. However, ITN, gravidity and malaria parasites are factors associated with anaemia (P-value < 0.05). The absence of IPT might result to the high prevalence of malaria in the pregnant women. More than half of the respondents had two or more pregnancies with successful delivery. The primigravidae had the highest malaria parasitaemia. Most of the pregnant women reported at ANC during the second trimester. The subjects under study had little knowledge on the effect of malaria and anaemia on their health.

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6.2 RECOMMENDATION

- Malaria and anaemia can be controlled effectively with administration of IPT, folic acid tablets including balanced diet during pregnancy.

- Community Health Education should be provided to positively influence the knowledge and attitudes of pregnant and non-pregnant women of child-bearing age to malaria and anaemia, including early antenatal registration.

- Assessment of blood transfusion cases, Body Mass Index (BMI) and other causes of anaemia apart from malaraia are strongly recommended.

- At least two stool samples should be used for the determination of intestinal nematodes.

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APPENDICES

APPENDIX 1

MALARIA AND ANAEMIA IN PREGNANT AND NON-PREGNANT WOMEN OF CHILD-BEARING AGE AT THE UNIVERSITY HOSPITAL- KNUST, KUMASI

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QUESTIONNAIRE

ID: ……

Age: ……...

Occupation: ……

Level of education

[ ] Illiterate [ ] Tertiary level

[ ] Primary education [ ] Non formal education

[ ] JSS/O – level

[ ] SSS/A – level

Gestation Period [ ] 1st trimester [ ] 2nd trimester [ ] 3rd trimester

Knowledge of malaria on pregnancy

1. Do you have any knowledge on malaria? Yes [ ] No [ ]

2. Does malaria have any effect on mother during pregnancy? Yes [ ] No [ ]

3. If yes what effect do you know of? ……

4. Does malaria have any effect on the unborn baby during pregnancy? Yes [ ] No [ ]

5. If yes what effect do you know of? ……...

……..

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Malaria in and outside pregnancy

1. How many pregnancies have you had? .....

2. How many children do you have? ……

3. Have you have malaria before? Yes [ ] No [ ]

4. Have you had malaria both within and outside pregnancy before? Yes [ ] No [ ]

5. If yes which one was more serious? [ ] during pregnancy [ ] outside pregnancy

6. What periods in pregnancy did you develop the severest form of the disease?

[ ] 1st trimester [ ] 2nd trimester [ ] 3rd trimester

Assessment of antimalarial programmes

1. Do you attend ANC regularly? Yes [ ] No [ ]

2. If no why? ……..

3. Are you given any counseling on malaria and anaemia prevention in pregnancy on any of your visits to the ANC? Yes [ ] No [ ]

4. Do you follow the malaria and anaemia preventive method being given at the ANC?

   Yes [ ] No [ ]

5. If no why? ……..
   ……

6. Do you use any anti-malarial drug? Yes [ ] No [ ]

7. Do you use bed net? Yes [ ] No [ ]

8. If yes which type do you use? ……..
   ……

9. Do you use mosquito repellent? Yes [ ] No [ ]

10. If yes which type do you use? .....
    ……

Laboratory Result

11. Blood film for malaria parasites: ……

12. Hb Level: ……

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APPENDIX 2

MATERIALS USED FOR THE STUDY

2.2 Reagents:

i. Giemsa stain

ii. Giemsa buffer

iii. EDTA

iv. Methanol

v. Antiseptic (70% isopropyl alcohol)

vi. Disinfectant (Izal)

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2.3 Equipment:

i. Binocular microscope (with 10×, 40×, and 100× objectives)

ii. Haematology analyzer

iii. Tourniquet (latex-free)

iv. Gauze (sterile)

v. Safety Engineered Vacutainer Needles (sterile)

vi. Gloves

vii. Blood Collection Tubes (EDTA tubes)

viii. Bio-hazardous Waste Containers

ix. Bandages

x. Timer

xi. Cover slips (22mm × 22mm)

xii. Beakers

xiii. Test tube racks

xiv. Permanent marker/Indelible ink pen

xv. Microscope slides (75mm × 22mm)

xvi. Coplin jar

xvii. RDT Kit for *P. falciparum*

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APPENDIX 3

Protocol for Giemsa staining

Materials Required:

Giemsa stain

Buffered Water, pH 7.1-7.2

Or buffered Saline, pH 7.1-7.2

Method:

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1. Immediately before use, dilute the Giemsa stain as required:

   3% solution for 30 minute staining

   Measure 50 ml of buffered water (or saline) pH 7.1-7.2. Add 1.5 ml of Giemsa stain and mix gently. The stain can be measured using a dry graduated plastic bulb pipette or a small volume (2 ml) plastic syringe.

   10% solution for 10 minutes staining

   Measure 45 ml of buffered water, pH 7.1-7.2 in a 50 ml cylinder. Add 5 ml of Giemsa stain (to 50 ml mark) and mix gently.

2. Place the slides face downwards in a shallow tray supported on two rods, in a Coplin jar, or in a staining rack for immersion in a staining trough. Thick blood films must be thoroughly dried and thin blood films must be fixed (methanol for 2 minutes).

3. Pour the diluted stain into the shallow tray, Coplin jar, or staining trough. Stain as follows:

   30 minutes if using 3% stain solution

   10 minutes if using 10% stain solution

4. Wash the stain from the staining container using clean water (need not be distilled or buffered)
5. Wipe the back of each slide clean and place it in a draining rack for the preparation to air-dry.
6. When the film is completely dry, apply a drop of immersion oil to an area of the film which appears mauve coloured (usually around the edges)
7. Spread the oil to cover an area about 10 mm in diameter
8. Preliminary scanning with the 10× and 40× objectives
9. Select the area that is well stained and not too thick. Change to the 100× objective.
10. Examine for malaria parasites and malaria pigment.
11. Report the maximum number of parasites (trophozoites, schizonts, and gametocytes) and also whether malaria pigment is present in white cells (always mention when the pigment is in the neutrophils).

Reporting:

12. If no parasites are found after examining 100 fields, report the film as:

    Malaria thick film: NPF (No Parasites Found)

13. The following plus sign scheme can be used to report parasite numbers:

Parasite Number		**Report**
1 – 10	per 100 high power fields …….	+
11 – 100	per 100 high power fields …….	++
1 – 10	in every high power field ……...+++	
>10	in every high power field ……...++++	

APPENDIX 4

CASSETTE MALARIA RDT TECHNIQUE

The device used was paracheck Pf cassette device which detects *P. falciparum* HRP 2 antigen in whole blood.

1. Immediately before use open the pouch of the cassette
2. Cleanse the finger with the swab provided
3. Prick the finger with the lancet provided
4. Collect blood sample with the loop provided
5. Blot the blood onto sample pad A
6. Add 2 drops of buffer to buffer port B
7. Read the results at the end of 15 minutes

Positive test for *P.falciparum*: Pink line appears in both the C (Control) and T (Test) viewing windows.

Negative test for *P.falciparum*: Pink line appears in the C (Control) viewing window only.

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APPENDIX 5

DIRECT WET MOUNT TECHNIQUE, SALINE PREPARATION

1. With a marker the study identification number is written at one end of the slide and a drop of physiological saline is placed in the center of the slide.
2. With a wooden applicator stick, a small portion of the stool sample (approximately 2 mg which is about the size of a match head) is picked and added to the drop of saline and thoroughly emulsified to make a thin uniform saline suspension-not to thick that fecal debris may obscure organisms, and not too thin that blank spaces may be present
3. The suspension is carefully covered with a cover slip in a way as to avoid air bubbles
4. The slide is then placed on the microscope stage, and the preparation is examined systematically under the low power (×10) objective so that the entire cover slip area is scanned for parasite ova, cysts, larvae and trophozoites.
5. When organisms or suspicious objects are seen, the high power (×40) objective is used to see more the detailed morphology of the object for confirmation.

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