FACTORS ASSOCIATED WITH ASYMPTOMATIC MALARIA PARASITAEMIA AMONG WOMEN ATTENDING ANTENATAL CLINIC IN GENERAL HOSPITAL NASSARAWA-EGGON, NASARAWA STATE, NORTH CENTRAL NIGERIA

BY

Samuel Ebuga, EMIASEGEN

DEPARTMENT OF COMMUNITY MEDICINE
AHMADU BELLO UNIVERSITY
ZARIA - NIGERIA

JANUARY, 2016

FACTORS ASSOCIATED WITH ASYMPTOMATIC MALARIA PARASITAEMIA AMONG WOMEN ATTENDING ANTENATAL CLINIC IN GENERAL HOSPITAL NASSARAWA-EGGON, NASARAWA STATE, NORTH CENTRAL NIGERIA

BY

Samuel Ebuga, EMIASEGEN,
MPH/N-FELTP/MED/42421/2012-2013

A DISSERTATION SUBMITTED TO
THE SCHOOL OF POSTGRADUATESTUDIES
AHMADU BELLO UNIVERSITY ZARIA,
IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR
THEAWARD OF MASTERS IN PUBLIC HEALTH FIELD EPIDEMIOLOGY
AND LABORATORY TRAINING PROGRAMME
DEPARTMENT OF COMMUNITY MEDICINE
AHMADU BELLO UNIVERSITY ZARIA

JANUARY, 2016

DECLARATION

I declare that the work in this dissertation entitled "Factors associated with asymptomatic malaria parasitaemia among women attending antenatal clinic in General Hospital Nassarawa-Eggon, Nasarawa State," was done by me under the supervision of Dr F.J. Giwa and Dr S. A. Ahmed. The information from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____ _____

Samuel Ebuga, EMIASEGEN Date

CERTIFICATION

This dissertation titled **"FACTORS ASSOCIATED WITH ASYMPTOMATIC MALARIA PARASITAEMIA AMONG WOMEN ATTENDING ANTENATAL CLINIC IN GENERAL HOSPITAL NASSARAWA-EGGON, NASARAWA STATE, NORTHCENTRAL NIGERIA"** by Samuel Ebuga, EMIASEGEN, meets the regulations governing the award of the degree of Masters in Public Health Field Epidemiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Dr. F.J. Giwa — Date
MBBS, FMCPath,
Chairman Supervisory Committee

_____ _____

Dr. S. A. Ahmed — Date
MBBS, MPH, FMCPath, FICS, IFCAP
Member, Supervisory Committee

_____ _____

Professor M. N. Sambo — Date
MBBS, FWACP, MIAD
Head of Department
Department of Community Medicine

_____ _____

Professor Kabiru Bala — Date
Dean, School of Postgraduate Studies

DEDICATION

This work is dedicated to God Almighty (the author and accomplisher of all that has been and all that will come to be) and to the memory of my late father Mr. Jeremiah Emiasegen.

ACKNOWLEDGEMENT

All thanks be to God Almighty for his endless mercy, inspiration and guidance through all my endeavours.

Special thanks to my supervisors (Academic and Programme) Dr. F. J. Giwa, Dr. S.A. Ahmed and Prof. A.T. Olayinka for their assistance and guidance all through the course of this work.

Special thanks to my research assistants and all the staff of General Hospital Nassarawa-Eggon that offered their services in the course of this research; Mrs Bolanle Ipaye, Mr Agbutun A. Micah, Mrs Helen O. Abu, Mrs Patience Kuje, Mr Peter Anzaku, and Miss Blessing Emmanuel.

Special appreciation to my Directors at Nasarawa State Hospital Management Board; Adamu Y. Ohagenyi and Garba Rosha.

Sincere thanks to my Wife Mrs. Fidelia Omozusi Emiasegen for being with me.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

ANC - Antenatal Clinic

CI - Confidence Intervals

CSA - Chondroitin Sulphate A

HRP2 - Histidine Rich Protein2

IPT - Intermittent Preventive Treatment

IRS - Indoor Residual Spray

IUGR - Intra Uterine Growth Restriction

LBW - Low Birth Weight

LGA - Local Government Area

LLIN - Long Lasting Insecticidal Net

NMPS - No Malaria Parasites Seen

OIC - Organisation of Islamic Countries

PCR - Polymerase Chain Reaction

PCV - Packed Cell Volume

PLDH - Plasmodium Lactate Dehydrogenase

RBC - Red Blood Cells

RBM - Roll Back Malaria

RDT - Rapid Diagnostic Tests

WHO - World Health Organisation

ABSTRACT

Asymptomatic malaria parasitaemia in pregnancy is a major public health challenge responsible for significant morbidity and mortality in endemic areas. In areas with stable malaria transmission like Nigeria, the vast majority of infections with *Plasmodium falciparum* in pregnancy remain asymptomatic, undetected and untreated with the attendant major impacts on the mother and the unborn fetus. The aim of this study was to determine the prevalence of asymptomatic malaria parasitaemia and its associated factors among women attending antenatal clinics (ANC) in a secondary health facility.

The study was conducted at the General Hospital, Nassarawa-Eggon, Nasarawa State, from June to August, 2014. Two hundred and forty-two pregnant women were recruited after obtaining an informed consent and a structured questionnaire was administered to each participant. CareStart^{TM} Rapid Diagnostic Test (RDT) kits and two thin and thick blood films were used to identify malaria parasites and estimate density. Haemoglobin levels were estimated using the packed cell volume (PCV) technique.

A total of 242 pregnant women participated in this study. About half of the women, (48.8%) were in the reproductive age group of 25 – 34 years,(65. 3%)were civil servants,(34. 3%) had a primary level of education and (63.2%) were multigravidae. The malaria specie that was identified in the area was *Plasmodium falciparum.* The percentage prevalence for malaria parasitaemia was 22. 7% by microscopy and 25.6% by RDT screening. Age below 25years and nonusage of LLIN were significantly associated with malaria parasitaemia while primigravidae and anaemia were not.

The level of asymptomatic malaria parasitaemia revealed in this study was high. Younger age of less than 25 years had highest risk of malaria parasitaemia. Failure to use LLIN is associated with an increased risk of malaria infection. Malaria parasitaemia can be responsible for anaemia in pregnancy and mother to child transmission of malaria. The performance of RDT for malaria screening in this study is comparable with Microscopy as the Gold Standard for use in our health facility. The administration of IPT should be intensified and routine diagnosis of malaria infection should be introduced as part of antenatal care strategy in our health facilities.

Keywords: Asymptomatic malaria parasitaemia, pregnant women, LLIN, Nassarawa-Eggon, Nigeria

CHAPTER ONE

INTRODUCTION

1. 1 Background

Malaria is a common parasitic disease, transmitted mainly by female Anopheles mosquitoes. Globally, 125 million women and approximately half of the world's population are at risk of malaria every year.[1] Most malaria cases and deaths occur in sub-Saharan Africa.[2] However, Asia, Latin America, and to a lesser extent the Middle East and parts of Europe are also affected. In 2014, 97 countries and territories had ongoing malaria transmission.[3] Seventy percent of pregnant women in Nigeria suffer from malaria with maternal and foetal complications.[4] Among the different species of *Plasmodium* parasites, *Plasmodium falciparum* is the most prevalent endemic species within the Nigeria sub-region and the most deadly.[5]

An increased risk of malaria during pregnancy was observed over 60 years ago,[6] and besides young children, pregnant women remain the main high risk group for malaria in endemic areas.[7] Frequency and severity of malaria are greater in pregnant women, than in non-pregnant women,[8] and causes serious adverse effects including abortion, low birth weight and maternal anaemia.[9] Incidentally malaria infection is more rampant among the primigravidae and secundigravidae than the Multigravidae.[10]

In areas of high or moderate transmission, most malaria infections in pregnant women are asymptomatic and infected women therefore do not present for treatment.[11]The clinical consequences of asymptomatic malaria may vary across different epidemiological settings and are not fully understood.[12] On the other hand, asymptomatic parasitaemia provides a reservoir for transmission and may be a precursor in the progression to symptomatic disease.[12]The presence of

parasites in peripheral blood without symptoms (asymptomatic malaria) is common in hyper-endemic areas, and is associated with chronic anaemia and placental sequestration.[10]The presentation of malaria during pregnancy varies according to the pre-existing immunity of the mother.[5] Women living in areas of low transmission have little immunity to malaria which can cause severe syndromes, such as cerebral malaria and pulmonary oedema. In contrast, those who live in areas of stable malaria transmission enjoy greater immunity and experience fewer symptoms during episodes of malaria; although they commonly develop severe anaemia as a consequence of the infection.[13]

National Malaria Control Programmes are geared towards the protection of pregnant women living in malaria endemic zones because of their reduced immunity[14]causing almost 25% of maternal deaths each year.[5] A number of factors influence the prevalence of malaria in pregnant women, including maternal age, use of prophylactic antimalarias, level of pre-pregnancy immunity, intensity and stability of malaria transmission in the region.[5]

1. 2 Problem Statement

Malaria remains a major cause of morbidity and mortality in endemic areas despite all efforts aimed at its control. Its transmission is intense and occurs all year round in Nigeria. In areas with stable malaria like Nigeria, the vast majority of infestations with *Plasmodium falciparum* in pregnancy remain asymptomatic, undetected and untreated with the attendant major impacts on the mother and the unborn fetus.[15]

Each year in sub-Saharan Africa, where 80-90% of world malaria cases occur, approximately 19-24 million women are at risk of malaria and its adverse consequences during

pregnancy.[1,2]According to the World Health Organization (WHO), malaria accounts for over 10,000 maternal and 200,000 neonatal deaths per year.[5]

A study by Roll Back Malaria (RBM) Partnership revealed that 238 million malaria cases, nearly half of malaria cases worldwide, are found in member states belonging to the Organization of the Islamic Cooperation (OIC). According to the report, 12 OIC countries are among the 20 most malaria-affected nations worldwide that account for nearly 80 percent of global cases, including Nigeria, Uganda, Sudan, Niger, Senegal, Cote d'Ivoire and Cameroon.[17] The leading position of Nigeria on the list clearly showed the challenge of malaria in Nigeria.

The principal impact of malaria infection is due to the presence of parasites in the placenta causing maternal anaemia (potentially responsible for maternal death when severe) and low birth weight.[18]Frequency and severity of malaria are greater in pregnant women, than in non-pregnant women.[8]Approximately 25 million pregnant women are at risk of *Plasmodium falciparum* infection every year, and one in four women has evidence of placental infection at the time of delivery.[(17,18)]Although IPT is given during ANC, routine screening for malaria has not been included in the antenatal care policy in Nigeria. Although Nasarawa State is among the States in Nigeria where the strategy on malaria reduction is being implemented, no study has been done in this locality to examine the prevalence and factors associated with malaria parasitaemia in pregnant women.

1. 3 Justification

Frequency and severity of malaria are greater in pregnant women, than in non-pregnant women[8]and causes serious adverse effects including abortion, low birth weight and maternal anaemia.[9] Approximately 25 million pregnant women are at risk of *Plasmodium falciparum*

infection every year, and one in four women has evidence of placental infection at the time of delivery.[17,18]

Despite considerable efforts to control malaria, it is still the most prevalent and devastating disease in tropical Africa with pregnant women and children below five years being the highest risk groups.[21] With the recent interest to scale up malaria control efforts by the federal government of Nigeria, the study of asymptomatic malaria parasitaemia among pregnant women will provide a data based additional information to guide malaria control efforts. Beside intermittent preventive therapy given at ANC visit, routine screening for malaria has not been included in the antenatal care policy in Nigeria and presently there is paucity of studies in Nigeria to examine the prevalence and factors associated with asymptomatic malaria parasitaemia in pregnant women.

Although, Nasarawa State is one of the states in Nigeria where the strategy on malaria reduction is being implemented, with Nassarawa-Eggon as one of the two LGAs in the state where the indoor residual spray programme has been implemented, no study has been done in this locality to examine the outcome, prevalence and associated factors of malaria parasitaemia in pregnant women. These investigations on the factors associated with asymptomatic malaria parasitaemia in pregnant women will serve to provide evidence based data that can be used to support malaria management and control efforts in the study area.

1.4 Research Questions

1. What is the prevalence of malaria parasitaemia in asymptomatic women attending antenatal clinic in General Hospital Nassarawa-Eggon?
2. What are the factors associated with asymptomatic malaria parasitaemia-among the women?
3. What is the correlation between parasite densities and degree of anaemia among women attending antenatal clinic in Nassarawa-Eggon General Hospital?
4. How effective is the use of RDT compared with Microscopy?

1. 5 Objectives

1. 5. 1 General Objective: To determine the prevalence of asymptomatic malaria parasitaemia and its associated factors among women attending antenatal clinics (ANC) in Nassarawa-Eggon General Hospital, Nasarawa State.

1. 5. 2 Specific Objectives:

1. To determine the prevalence of malaria parasitaemia in asymptomatic women attending ANC in General Hospital Nassarawa-Eggon.
2. To identify factors associated with asymptomatic malaria parasitaemia-among the women.
3. To determine the correlation between parasites densities and degree of anaemia among women attending ANC in Nassarawa-Eggon General Hospital, Nasarawa State.
4. To validate the use of RDT by comparing with the Gold Standard.

1. 5. 3 Scope of the Study:

The study covered women of child bearing age (15-49 years) residing in Nassarawa-Eggon LGA of Nasarawa State during a period of three months. It determined the prevalence of malaria parasitaemia in asymptomatic women attending ANC mainly in General Hospital Nassarawa-Eggon, Nasarawa State.

CHAPTER TWO
LITERATURE REVIEW

2. 1 Public Health Importance of Malaria

Malaria in pregnancy is a major contributor to adverse maternal and perinatal outcome. It is an important cause of anaemia, miscarriages, intrauterine growth restriction, low birth weight, still birth and other pregnancy-related complications.[4] Malaria poses enormous public health burden worldwide.[7]Several studies have reported that 125 million women worldwide and 25-50 million African women who get pregnant annually are at risk of malaria.[20,18]Sub-Saharan Africa records each year about thirty-two million pregnant women living in areas of high transmission of *Plasmodium falciparum* causing malaria.[23]

Ninety percent of global malaria burden occur in Africa South of Sahara where malaria in pregnancy has been most evaluated.[24] Seventy percent of pregnant women in Nigeria suffer from malaria with maternal and foetal complications.[4] In hyperendemic areas like Nigeria, malaria is a common cause of anaemia in pregnancy in both immune and non-immune individuals and is aggravated by poor socioeconomic circumstances.[10]Most pregnant women with malaria infestation are asymptomatic thus are undetected and untreated.[25]Depending on the endemicity of malaria in an area, it can be expected that 1-50% of pregnant women may carry malaria parasitaemia, especially in the placenta, without noticing it.[26] At this period, unfortunately, the subclinical infection still poses a great danger to both the mother and the foetus.[14]

Human malaria is caused by five species of Plasmodia: *P. falciparum*, *P. vivax, P. ovale, P. malariae and P. knowlesi.*[2,25] Among those infected, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (~20%).[28]*P. knowlesi* is a zoonotic species that causes malaria in macaques[27]and is mostly of limited public health importance.[29]The burden of malaria

in pregnancy is caused chiefly by *Plasmodium falciparum*, the most virulent of the Plasmodium parasites, especially in the sub-Saharan Africa.[30]

Pregnant women are more susceptible than the general population to malaria: they are more likely to become infected, suffer a recurrence, develop severe complications and to die from the disease.[31]Malaria contributes very significantly to maternal and fetal mortality with at least 10,000 maternal deaths per annum attributable in sub-Saharan Africa.[32]

Regardless of symptoms, the presence of plasmodium parasites in a pregnant woman's body will have a negative impact on her own health and that of her fetus.[31] Subclinical infection is common in areas where natural immunity is high (eg, sub-Saharan Africa), whereas symptomatic cases are more common in areas with low immunity (eg, the Asia-Pacific region, and South Africa).[33]

Malaria in pregnancy is different from the disease in the non-pregnant state.[31] The severity of malaria in pregnancy is thought to be due to general impaired immunity plus a diminution of acquired immunity to malaria in endemic areas. Placental malaria occurs where *Plasmodium falciparum*-infected erythrocytes accumulate in the intervillous space of the placenta but may be rare or absent in the peripheral circulation.

2. 2 Epidemiology

Understanding the epidemiology of malaria during pregnancy provides important insight into relevant immunological processes and facilitates decision on control strategies.[34]Producing good estimates of the global burden of malaria is difficult due to poor numerator (number of women affected by malaria in pregnancy) and denominator (population at risk) data. However globally, 125 million women are at risk of malaria every year.[1]In sub-Saharan Africa, in area most

burdened by malaria, the disease is thought to cause as many as 10,000 cases of malaria-related deaths in pregnancy, mainly due to severe maternal anaemia.[32] Infant mortality rates are very difficult to come by and lack of reliable data has been identified as a major problem in the planning of anti-malarial public health services.[31] Statistics in 2010 suggested that there were 655,000 malaria-related deaths globally of which 86% were in children under the age of five.[31]The number related to malarial infection in pregnancy is, however, not known.[35]

A recent review of studies, carried out in sub-Saharan Africa between 2000 and 2011, reports that prevalence of malaria in pregnant women attending antenatal clinics was 29. 5% in East and Southern Africa and 35.1% in West and Central Africa.[33]Further studies of mortality and morbidity in pregnant women resident in this region are required.[36]Malaria makes a large but unquantifiable contribution to low birth weight in infants in the developing world, a major cause of morbidity and mortality in infants and children.[31]

2. 3 Malaria Transmission

Malaria is transmitted when an infected mosquito takes a human blood meal and the *Plasmodium* sporozoites are transferred from the saliva of the mosquito into the capillary bed of the host.[5]Factors that influence mosquito breeding, such as temperature, humidity, and rainfall, affect malaria incidence.[37]In malaria endemic regions, individuals are constantly exposed to malaria parasites through the bites of infected female Anopheles mosquitoes.[14] This frequent exposure leads to the development of an effective anti-disease immunity to malaria, which prevents life-threatening parasite burdens and suppresses the pro-inflammatory responses which cause illness.[38]Few infections in healthy adults living in areas of high malaria transmission result in fever, and the same is true for semi-immune pregnant women.[20]

2. 4 Malaria in Pregnancy

Malaria in pregnancy is caused by plasmodium species, which include *Plasmodium falciparum, Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*.[5] The infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world.[39] Pregnant women are 3 times more likely to suffer from severe disease as a result of malarial infection compared with their non-pregnant counterparts, and have a mortality rate from severe disease that approaches 50%.[38,39]The problem of malaria in pregnancy was not described until the early 20th century.[42] Africa South of the Sahara bears 90% of the global malaria burden.[35]

During pregnancy, the acquired semi-immunity is able to keep the infection at an asymptomatic level in the majority of cases.[41,42] Depending on the endemicity of malaria in an area, it can be expected that 1-50% of pregnant women may carry malaria parasitaemia, especially in the placenta, without noticing it.[26] At this period unfortunately, the subclinical infection still poses a great danger to both the mother and the foetus.[14] The principal impact of malaria infection in pregnancy is due to the presence of parasites in the placenta causing maternal anaemia (potentially responsible for maternal death when severe) and low birth weight (LBW), a major predictor of infant and neonatal mortality.[9,43]

Malaria has been shown in many studies to worsen certain pregnancy outcomes. These include an increased incidence of anaemia and spontaneous abortions. Others include intrauterine growth restriction [IUGR], stillbirths, prematurity, low birth weight, fetal distress and congenital malaria.[4] The biological basis for these adverse outcomes has been extensively studied. Erythrocytes infected with *Plasmodium falciparum* accumulate in the placental bed. This is through adhesion of the infected erythrocytes to molecules of Chondroitin A present in the

placenta.[46] A prevalence of placental parasitaemia of between 10 and 45% in malaria endemic areas has been reported with significant *Plasmodium falciparum* dominance.[18,45]

Intra-placental parasitaemia has also been shown to increase with gestational age and with the highest risk of infection being in the second trimester,[20] and often extending to the immediate postpartum period.[46,47] The effect of malaria in pregnancy is worse in the first and second pregnancies compared to higher parities.[18,48] Acute infection with high levels of placental parasitaemia has been associated with preterm delivery,[51]while chronic placental parasitisation is associated with intrauterine growth restriction, lower maternal haemoglobin and severe anaemia.[50,51]

The greatest degree of placental infestation is seen in women who have the highest level of immunity, leading to milder maternal symptoms and a disproportionate increase in fetal complications.[40] It could be hypothesized, therefore, that although primigravidae may develop the clinical symptoms of malaria, women with higher immunity may not demonstrate symptoms, will not receive treatment, and will build a higher placental parasite burden.[5] Fetal complications result from this placental inflammation, as well as maternal anemia, and manifest as stillbirth, intrauterine growth restriction, and low-birth-weight neonates.[5] Low-birth-weight neonates, in turn, are at higher risk for neonatal and newborn death.[5]

It is also thought that infected erythrocytes collected in the placenta stimulate pancreatic β-cell production of insulin, leading to hyper-insulinaemia and hypoglycemia during infection.[5] This contributes to the severity of disease during pregnancy. Other maternal effects of malarial infection result from the "stickiness" of the infected erythrocytes that become trapped in small vessels, resulting in cerebral malaria, renal failure, and thrombocytopenia.[5] Case reports

confusing malaria infection with HELLP syndrome demonstrate the overlap in clinical and laboratory findings between the 2 diseases and the importance of proper diagnosis.[54]

2. 5 Susceptibility of Pregnant Women to Malaria

Pregnancy increases the frequency and severity of most infectious diseases but its effect on malaria seems worse.[18,48] Several theories have been put forward to explain this increased risk including changes to the cellular immune responses that otherwise should offer protection, and increased attractiveness of the pregnant woman to mosquitoes. The former is believed to result from the increased level of circulating maternal steroids in pregnancy.[55] This was the subject of the extensive research by Bouyou-Akotet et al in which they surmised that a sustained increase in cortisol level underlies the increased susceptibility of pregnant women to malaria.[56] Lindsay et al found that pregnant women attracted twice the number of anopheles mosquito compared to their non-pregnant counterparts.[57] This they believed may be connected to certain physiological and behavioural changes that occur in pregnancy including increased volume of exhaled air and release of volatile substances from their skin surfaces due to increased skin temperature associated with pregnancy.[57] These substances may be detected by the mosquitoes hence leading to increased attractiveness of the pregnant woman to mosquito.[55,56]

The susceptibility to malaria including its complications like anaemia is worse in the first and second pregnancies especially in young gravidaes.[48,57] Though many of the infections may be asymptomatic in women in endemic countries, this does not preclude placental parasitisation and its deleterious effects.[45,53,57] Up to 20% of pregnant women in endemic areas have asymptomatic parasitaemia and a recent study showed that about 40% of these will present with clinical malaria within a four week period.[53,57] Cerebral malaria, acute renal failure and severe hemolysis, are

complications of malaria that are rare in adults in endemic areas, which may be seen in pregnancy.[55]

Susceptibility to *Plasmodium* parasitaemia has been linked to the level of antibodies to placental sequestrated parasites.[18] These parasites preferentially adhere to chondroitin sulphate-A receptors (CSA) expressed by the syncytiotrophoblasts in the placenta.[60] Women in their first and second pregnancies are more susceptible as anti-adhesion antibodies against CSA binding parasites develop after successive pregnancies.[61] Incidentally malaria infection is more rampant among the primigravidae and secundigravidae than the Multigravidae.[10] The preferential susceptibility of these sets of pregnant women may be related to some evidence that immuno-suppression associated with pregnancy, occurs more in the first than subsequent pregnancies.[62] Previously, the depression of cell mediated immune response to *Plasmodium falciparum* antigens has been implicated in this phenomenon.[63] Age has also been implicated as epidemiological studies have shown that malaria in pregnancy is more prevalent in younger than older age groups.[9,62]

2. 6 Anaemia in Pregnancy

Anaemia in pregnancy has been associated with maternal morbidity and mortality and is a risk factor for low birth weight.[65]Anaemia is said to be the commonest medical condition in pregnancy with a prevalence of 50% worldwide.[66]Recent estimates in the developing countries including Nigeria put the prevalence at 60% in pregnancy and about 7% of the women are said to be severely anaemic.[64,65] Most of those affected are the primigravidas and grandmultiparas.[68] Excessive rapid destruction of red blood cells, bleeding during pregnancy and inadequate haematopoeisis are the three major causes.[15]Severemalaria parasitaemia appear to be a leading cause especially in our environment.[(67,68)]

Malaria causes increased haemolysis of parasitized red blood cells.[15] The degree of haemolysis depends on the burden of parasites.[71]Beside malaria, helminthiases as well as nutritional factors also contribute variously to anemia during pregnancy.[72] However, haemoglobin is the driving force for oxygen and nutrients for mother and fetus, therefore a reduction below acceptable levels can be detrimental to both. A hemoglobin concentration below 11.0 g/dl or packed cell volume (PCV) of less than 33. 0% is regarded as anemia during pregnancy by the World Health Organization (WHO).[73]

Although adults living in endemic areas acquire protective immunity against developing severe malaria, they become more susceptible especially when they become pregnant.[26] In areas with stable malaria like Nasarawa State of Nigeria, the vast majority of infestations with *Plasmodium falciparum* in pregnancy remain asymptomatic, undetected and untreated.

2. 7 Diagnosis of Malaria in Pregnancy

Making a diagnosis in pregnancy requires careful clinical examination and laboratory investigation. Whereas malaria could be over diagnosed in endemic areas,[72,73]in the non-endemic areas a high index of suspicion is usually required.[76] The gold standard for malaria parasite identification and quantification has been the microscopic examination of thick and fixed thin blood smears using Giemsa stain. In cases of anticipated low malaria parasite densities, care should be taken to maintain the pH of the stain around 7 and a freshly prepared stain achieves better results.[77]

Other techniques utilised to enhance microscopy include the acridine orange fluorescent technique,[78] which requires the use of fluorescent microscopy. There is also the antigen detecting rapid diagnostic tests (RDTs) which detect the histidine rich protein2 (HRP2) and Plasmodium

lactate dehydrogenase (pLDH) which are usually produced during the erythrocytes cycle. There is also Polymerase chain reaction (PCR) techniques which has been utilised to enhance malaria diagnosis in mixed infections and especially in patients with low parasites density.[79]

2. 8 Prevention and Control of Malaria in Pregnancy

The policy and strategies for Malaria control by the WHO hinges on Malaria prevention, diagnosis and treatment,[80] while the protection of pregnant women living in malaria endemic countries has been of particular interest to many National Malaria Control Programmes because of the reduction in immunity associated with pregnancy.[14] The reduction in cell-mediated immunity is a physiological response that allows foetal allograft retention, but it is thought to also interfere with resistance to various infectious diseases.[81]

The focus of malaria prevention during pregnancy has been the use of antimalarial chemoprophylaxis and the use of Long Lasting Insecticidal Nets (LLINs). Pregnant women on antimalarial chemoprophylaxis are at a reduced risk of the harmful effects of malaria,[82] because the chemoprophylaxis includes Intermittent Preventive Treatment (IPT), in which full dose of a drug is given at defined intervals. Although the use of LLINs has shown potential efficiency in the control of mosquito bites, Nwagha et al, however, identified that a pregnant woman cannot remain under the net for more than 8 hours a day; hence it does not give complete protection against malaria.[10] Agomo and Oyibo also identified non-usage of insecticidal spray as important risk factors associated with malaria infection during pregnancy.[14] But Indoor Residual Spraying (IRS) with insecticides is a powerful way to rapidly reduce malaria transmission.[16] Its full potential is realized when at least 80% of houses in targeted areas are sprayed and it is effective for 3–6 months, depending on the insecticide used and the type of surface on which it is sprayed.[16]

CHAPTER THREE
METHODOLOGY

3. 1 Study Area:

General Hospital Nassarawa-Eggon, Nasarawa State is the study site. The hospital is the main secondary health facility in the local government. The Local government has an area of 1,208 km² and a projected population of 194,580 based on the 2006 census. The LGA is centrally located in the State, and comprises of both urban and rural populace. Nassarawa-Eggon LGA is also one of the two LGAs in the state (the other one is Doma LGA) in which the indoor residual spray activity has been implemented.

The occupation of the people in Nassarawa-Eggon is mainly farming and agricultural businesses. The climate of Nassarawa-Eggon LGA falls within the tropical savannah (Aw) climate with two clearly marked seasons, wet and dry. It has a mean temperature of 15. 6ºC and 26.7ºC with an annual rainfall between 1317mm and 1450mm. 1t rains from April to October and the months of December to February experiences the northeast trade winds and thus the dry harmattan.[83] The onset of rains in April ushers in a noticeable decline in temperature. This continues to the cessation period by October ending when a further decline is made possible in November/December by the coming of the harmattan winds.[83]

The continuous transmission of malaria in the area is maintained by the abundance of pockets of water within the Rocky Mountains and the drainage facilities in Nassarawa-Eggon area.

General hospital Nassarawa-Eggon besides being the only secondary health care facility in the LGA, it is also the highest level of health facility in the area. The hospital has 26 beds and several departments/units. Services rendered include; clinical, laboratory, pharmacy, nursing, surgical, ambulatory and antenatal care (ANC) clinic with a ward for pregnant women.

The hospital has antenatal clinic attendance of around 20 and 25 pregnant women per week and a twice weekly antenatal clinic activities (the first ANC activity for each week is usually for those coming for booking while the second ANC activity for each week is for those on subsequent visit or appointment) during which Sulphadoxine Pyrimethiamine (SP) prophylaxis in pregnancy are received.

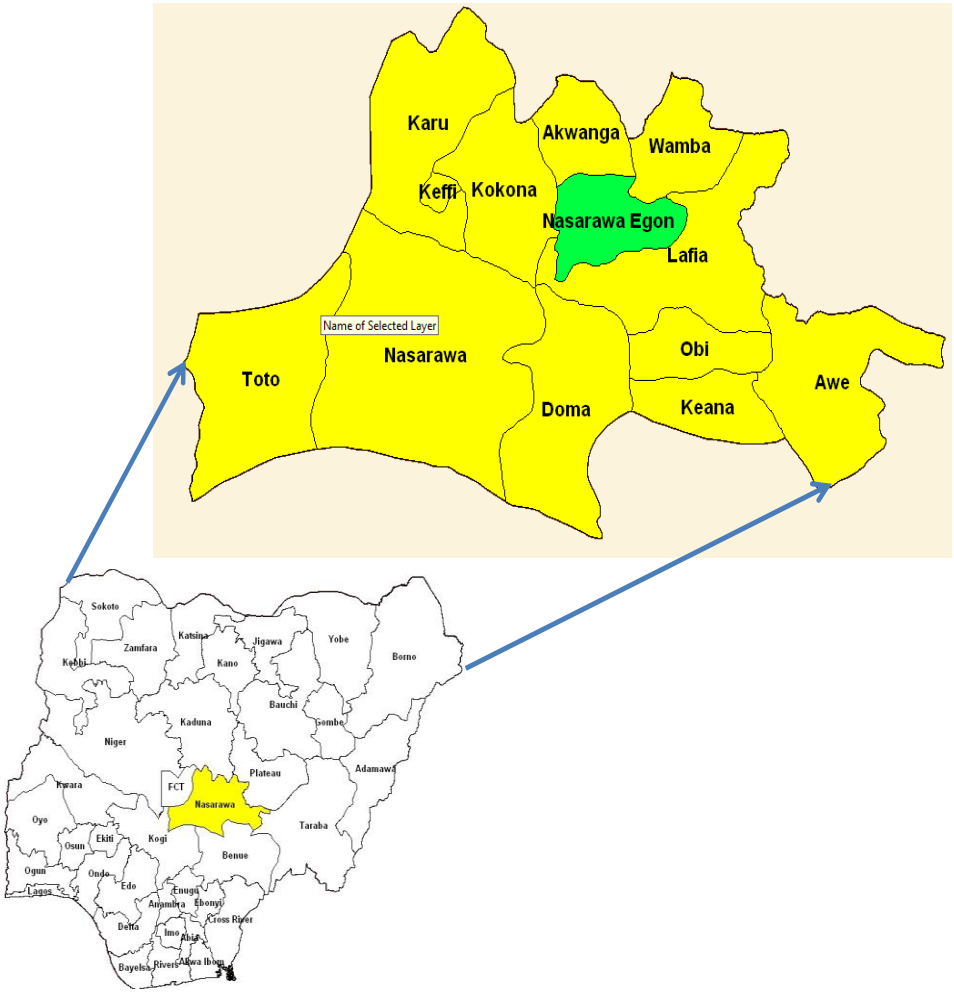

Figure 3.1: Map of Nigeria highlighting Nasarawa State in yellow and Nassarawa-Eggon LGA in green

3. 2 Study Design:

Across-sectional study was carried out from June to August, 2014.

3. 3 Study Population:

Are women attending antenatal clinic at the LGA General Hospital. An average of 25 women attend the antenatal clinic weekly in the health facility.

3. 3. 1 Inclusion:

Healthy, afebrile pregnant women, resident in Nassarawa-Eggon LGA who consented to participate were included in the study.

3. 3. 2 Exclusion Criteria:

Women on anti malaria therapy two weeks earlier were excluded from the study.

3. 4 Sample Size Determination:

The number of samples used for the study was 242. This was estimated using the formula for quantitative outcome by applying a prevalence of 14% for malaria parasitaemia from Minna, Niger State [84] at a precision of 0. 05.

Where,

n = the minimum sample size

Z_{α} = the standard normal deviate corresponding to a level of significance of 5%=1. 96

d = the desired level of precision (usually at 5% for single proportions) = 0. 05

p = prevalence of malaria parasitaemia among pregnant women from a previous study = 14%

Therefore, $n = \frac{Z_{\alpha}^2 pq}{d^2}$

$n = \frac{1.96^2 * 0.14 * 0.86}{0.05^2} = 185$

Adding 25% non-response rate

Where f = the non response rate

The sample size will be = n/1-f

= 185/1 – 0. 25 =185/0. 75 = 247

However, five of the participant were identified to have been enrolled twice and this was adjusted for, bring the sample size to 242.

3. 5 Sampling Technique:

Pregnant women were recruited consecutively as they presented for booking to the antenatal clinic for a period of three months.

3. 6 Study Instruments:

Interviewer administered questionnaire adopted[85]and modified was used to obtain information on socio-demographics and associated risk factors for malaria parasitaemia. Others are laboratory equipment and reagents which were used for sample collection, processing and analysis.

3. 6. 1 Questionnaire:

An interviewer trained administered structured questionnaire was used to obtain information on patients' socio-demographics and factors associated with asymptomatic malaria parasitaemia in

pregnant women attending antenatal clinic at the general hospital in Nassarawa-Eggon Local Government Area, Nasarawa State.

3. 6. 2 Laboratory Equipment's and Reagents:

These includes; Microscope, Slides, Slide rack, Giemsa staining reagents and the Rapid Diagnostic Test (RDT) Kits. The Giemsa staining technique was used with microscopy as the Gold Standard while RDT used worked with the principle of antigen detection of Histidine Rich Protein2 (HRP2) specific for *Plasmodium falciparum*.

3. 6. 2. 1 Sample Collection:

This was carried out by using EDTA bottles, sterile needles and syringes to collect 3mls of blood aseptically from the cubital fossa of the arms of the pregnant women on antenatal care who consented to participate in the study.

3. 6. 2. 2 Sample Processing:

The collected blood samples were screened immediately for malaria parasitaemia using CareStart^{TM} Rapid Diagnostic Test (RDT) kits. Thick and thin blood films were also prepared immediately on the same slides for parasites detection and density estimation. For the thick films, 12 µl of blood was spread over a diameter of 15 mm, while 2 µl of blood was used for the thin films and the slides were labeled appropriately and air dried. The thin films were fixed in absolute methanol for 1-2 sec, stained with 3% Giemsa for 30minutes and air dried. The thick blood film was stained after 24hrs with 3% Giemsa stain solution at pH 7. 2.

3. 6. 2. 3 Malaria Parasites Detection and Estimation:

Stained slides were read by two trained microscopists. The slides were viewed using the x100 objective lens with immersion oil. Parasites present were identified and densities were estimated by counting against 200 leucocytes and multiplying it by 40 to get 8000. Those without parasites were indicated as; No Malaria Parasites Seen (NMPS) while density estimation was carried out using the formula:

$$\text{Number of Parasites/}\mu\text{ blood} = \frac{\text{Number of parasites x 8000 leucocytes}}{\text{Number of leucocytes counted}}$$

Discrepancies were calculated using the formula;

$$\%\ \text{Discrepancy} = \frac{\text{Count1} - \text{Count2}}{\text{Mean of Counts 1\&2}} \times 100$$

3. 6. 2. 4 Haemoglobin Level Estimation:

Haemoglobin levels were estimated using the packed cell volume (PCV) technique. The haematocrit tube was filled with blood and sealed with plastacin. The tubes were placed in the haematocrit centrifugate and spun at 5000 revolution per minute for 5minutes by haematocrit technique.[86] The PCV was then read using the Hawksley microhaematocrit reader and recorded accordingly.

3.6.2.5 Measurement of Variables:

The study variables were the dependent and independent variables: The dependent variables were the presence of parasitaemia and parasites density. The parasites densities were graded and measured as follows; low parasites density (1-999), moderate parasites density (1000-9999), high parasites density (≥10000).The independent variables were the socio-demographics and associate factors and were extracted and measured from the data collected.

3. 7 Data Entry:

Completed questionnaires and laboratory samples results were reviewed prior to electronic data entry. Several consistency checks were performed to exclude incomplete, inaccurate and inconsistent data entry.

3. 8 Data Management:

Data entry and cleanup was performed using Epi-info software version 3. 5. 3. Electronic data backup was created after data entry before statistical analysis.

3. 8. 1 Statistical Analysis:

Univariate data analysis was done to obtain frequencies and proportions. Bivariate data analyses was done to determine the relationship between malaria parasitaemia and parasites density with factors associated with asymptomatic malaria parasitaemia in pregnant women on antenatal care. The association between malaria parasitaemia and anaemia among the pregnant women was also determined. The levels of significance of the analysis were determined at 95% confidence intervals. Factors that were significant with bivariate analyses were subjected to unconditional logistic regression.

3. 9 Ethical Considerations:

Ethical clearance to carry out the study was obtained from the Ethical Research Committee at the Nasarawa State Ministry of Health [Ref: S/MOH/843/2014] (Appendix 1). Informed written consent were obtained from each of the study participants. Confidentiality of the participants and information provided were assured and maintained throughout the study period.

3. 10 Limitations:

The study is limited by the fact that it was carried out in a secondary health facility and there are other levels of health facilities (Primary and Tertiary). It may therefore be difficult to generalize the study to the whole population.

CHAPTER FOUR

RESULTS

Table 4.1: Characteristics of Women on ANC in General Hospital, Nassarawa-Eggon, Nasarawa State (n =242)

Characteristics	Frequency (n)	Percentage (%)
Age Group		
15 - 24	104	43. 0
25 – 34	118	48. 8
≥ 35 years	20	8. 3
Occupation		
Civil servant	158	65. 3
House wife	19	7. 9
Business	53	21. 9
Student	12	5. 0
Educational Level		
None	76	31. 4
Primary	83	34. 3
Secondary	65	26. 9
Tertiary	18	7. 4
Gravidity		
Primigravidae	89	36. 8
Multigravidae	153	63. 2
RDT Screening		
Positive	62	25. 6
Negative	180	74. 4
Microscopy		
Positive	55	22. 7
Negative	187	77. 3

A total of 242 pregnant women participated in this study. About half of the women (48.8%) were in the reproductive age group of 25 – 34 years,(65.3%) were civil servants, (34. 3%) had a primary level of education and (63.2%) were multigravidae. The use of Microscopy and RDT screening technique as identified in this study is comparable. The percentage prevalence for malaria parasitaemia was 22. 7% by microscopy and 25.6% by RDT screening (Table 4.1).

Table 4.2: Variables associated with malaria parasitaemia among women on ANC in General Hospital Nassarawa-Eggon, Nasarawa State (n =242)

Variables	*P. falciparum* Parasitaemia Positive n (%)	Negative n (%)	OR	95%C. I.	p-value
Age Group					
< 25	38 (36. 5)	66 (63. 5)	2. 7	1. 4 – 5. 0	< 0. 01
≥ 25	24 (17. 4)	114 (82. 6)			
Occupation					
Civil Servant/Student	50 (294)	120 (70. 6)	2. 1	1. 0 – 4. 2	0. 04
House Wife/Business	12 (16. 7)	60 (83. 3)			
Use of LLIN					
No	19 (45. 2)	23 (54. 8)	3. 1	1. 6 – 6. 2	< 0. 01
Yes	43 (21. 5)	157 (78. 5)			
Had Blood Transfusion					
Yes	6 (31. 6)	13 (68. 4)	1. 4	0. 5 – 3. 8	0. 53
No	56 (25. 1)	167 (74. 9)			
History of Miscarriage					
Yes	10 (19. 2)	42 (80. 8)	0. 6	0. 3 – 1. 4	0. 23
No	52 (27. 4)	138 (72. 6)			
Informed about malaria prevention					
Yes	61 (25. 8)	175 (74. 2)	1. 7	0. 2 – 15. 2	0. 61
No	1 (16. 7)	5 (83. 3)			
Trimester					
First Trimester	13 (18. 6)	57 (81. 4)	0. 6	0. 3 – 1. 1	0. 11
Other Trimester	49 (28. 5)	123 (71. 5)			
Gravidity					
Primigravidae	32 (36. 0)	57 (64. 0)	2. 3	1. 3 – 4. 1	< 0. 01
Multigravidae	30 (19. 6)	123 (80. 4)			
Education Level					
None/Primary	44 (27. 5)	116 (72. 5)	1. 3	0. 7 – 2. 5	0. 35
Secondary/Post Sec.	18 (22. 0)	64 (78. 0)			
Haemoglobin Level					
Low Level (< 11. 0 g/dl)	41 (32. 5)	85 (67. 5)	2. 2	1. 2 - 4. 0	0. 01
Normal Level (≥11. 0 g/dl)	21 (18. 1)	95 (81. 9)			

Age, Occupation, non-usage of LLIN, Primigravidae and low haemoglobin level (anaemia) were significantly associated with malaria parasitaemia (Table 4.2).

Table 4.3: Malaria parasites density among women on ANC in General Hospital Nassarawa-Eggon, Nasarawa State (n =242)

Variables	NMPS n(%)	Low n (%)	Moderate n (%)	High n (%)
Age Group				
15 - 24	69 (66. 3)	19 (18. 3)	13 (12. 5)	3 (2. 9)
25 - 34	98 (83. 1)	8 (6. 8)	12 (10. 2)	0 (0. 0)
≥ 35years	20 (100. 0)	0 (0. 0)	0 (0. 0)	0 (0. 0)
Occupation				
House Wife	18 (94. 7)	1 (5. 3)	0 (0. 0)	0 (0. 0)
Civil Servant	119 (75. 3)	19 (12. 0)	18 (11. 3)	2 (1. 3)
Business	42 (79. 2)	7 (13. 2)	4 (7. 5)	0 (0. 0)
Student	8 (66. 7)	1 (8. 3)	3 (25. 0)	0 (0. 0)
LLIN Use				
Yes	162 (81. 0)	22 (11. 0)	14 (7. 0)	2 (1. 0)
No	25 (59. 5)	5 (11. 9)	11 (26. 2)	1 (2. 4)
Had Blood Transfusion				
Yes	15 (78. 9)	3 (15. 8)	1 (5. 3)	0 (0. 0)
No	172 (77. 1)	24 (10. 8)	24 (10. 8)	3 (1. 3)
Trimester				
First trimester	60 (85. 7)	4 (5. 7)	6 (8. 6)	0 (0. 0)
Second trimester	95 (72. 0)	19 (14. 4)	16 (12. 1)	2 (1. 5)
Third trimester	32 (80. 0)	4 (10. 0)	3 (7. 5)	1 (2. 5)
Gravidity				
Primigravidae	61 (68. 5)	14 (15. 7)	12 (13. 5)	2 (2. 2)
Multigravidae	126 (82. 4)	13 (8. 5)	13 (8. 5)	1 (0. 7)
History of miscarriage				
Yes	43 (82. 7)	3 (5. 8)	6 (11. 5)	0 (0. 0)
No	144 (75. 8)	24 (12. 6)	19 (10. 0)	3 (1. 6)
Haemoglobin level				
Low Level (< 11. 0 g/dl)	90 (71. 4)	19 (15. 1)	16 (12. 7)	1 (0. 8)
Normal Level (≥ 11. 0 g/dl)	97 (83. 6)	8 (6. 9)	9 (7. 8)	2 (1. 7)

Higher parasites density were identified among age group 15-24years, civil servant, women who do not use LLIN, who never had blood transfusion, those in the second trimester, Primigravidae, women who had no history of miscarriage and those with low haemoglobin level (Table 4.3).

Table 4.4: Factors associated with malaria parasitaemia among women on ANC in General Hospital, Nassarawa-Eggon, Nasarawa State (n=242)

Variables	Malaria Parasitaemia n (%)	NMPS n (%)	OR	95%C. I.	p-value
Age Group					
< 25	13 (12. 7)	89 (87. 3)	0. 3	0. 2 – 0. 7	< 0. 01
≥25	42 (30. 0)	98 (70. 0)			
Occupation					
House wife/Students	43 (25. 3)	127 (74. 7)	1. 7	0. 8 – 3. 4	0. 14
Civil servants/Business	12 (16. 7)	60 (83. 3)			
Use of LLIN					
Yes	17 (40. 5)	25 (59. 5)	2. 9	1. 4 – 5. 9	< 0. 01
No	38 (19. 0)	162 (81. 0)			
Had Blood Transfusion					
Yes	4 (21. 1)	15 (78. 9)	0. 9	0. 3 – 2. 8	0. 86
No	51 (22. 9)	172 (77. 1)			
History of Miscarriage					
Yes	9 (17. 3)	43 (82. 7)	0. 7	0. 3 – 1. 4	0. 29
No	46 (24. 2)	144 (75. 8)			
Trimester					
First Trimester	10 (14. 3)	60 (85. 7)	0. 5	0. 2 – 1. 0	0. 05
Other Trimester	45 (26. 2)	127 (73. 8)			
Gravidity					
Primigravidae	28 (31. 5)	61 (68. 5)	2. 1	1. 1 – 3. 9	0. 01
Multigravidae	27 (17. 6)	126 (82. 4)			
Education Level					
None/Primary	38 (23. 8)	122 (76. 3)	1. 2	0. 6 – 2. 3	0. 60
Secondary/Post Sec.	17 (20. 7)	65 (79. 3)			
Haemoglobin Level					
Low Level (< 11. 0 g/dl)	36 (28. 6)19	90 (71. 4)	2. 0	1. 1–3. 8	0. 02
Normal Level (≥11. 0 g/dl)	(16. 4)	97 (83. 6)			

Age < 25years, non-usage of LLIN, Primigravidae, and low haemoglobin level (anaemia) were significantly associated with malaria parasitaemia (Table 4.4).

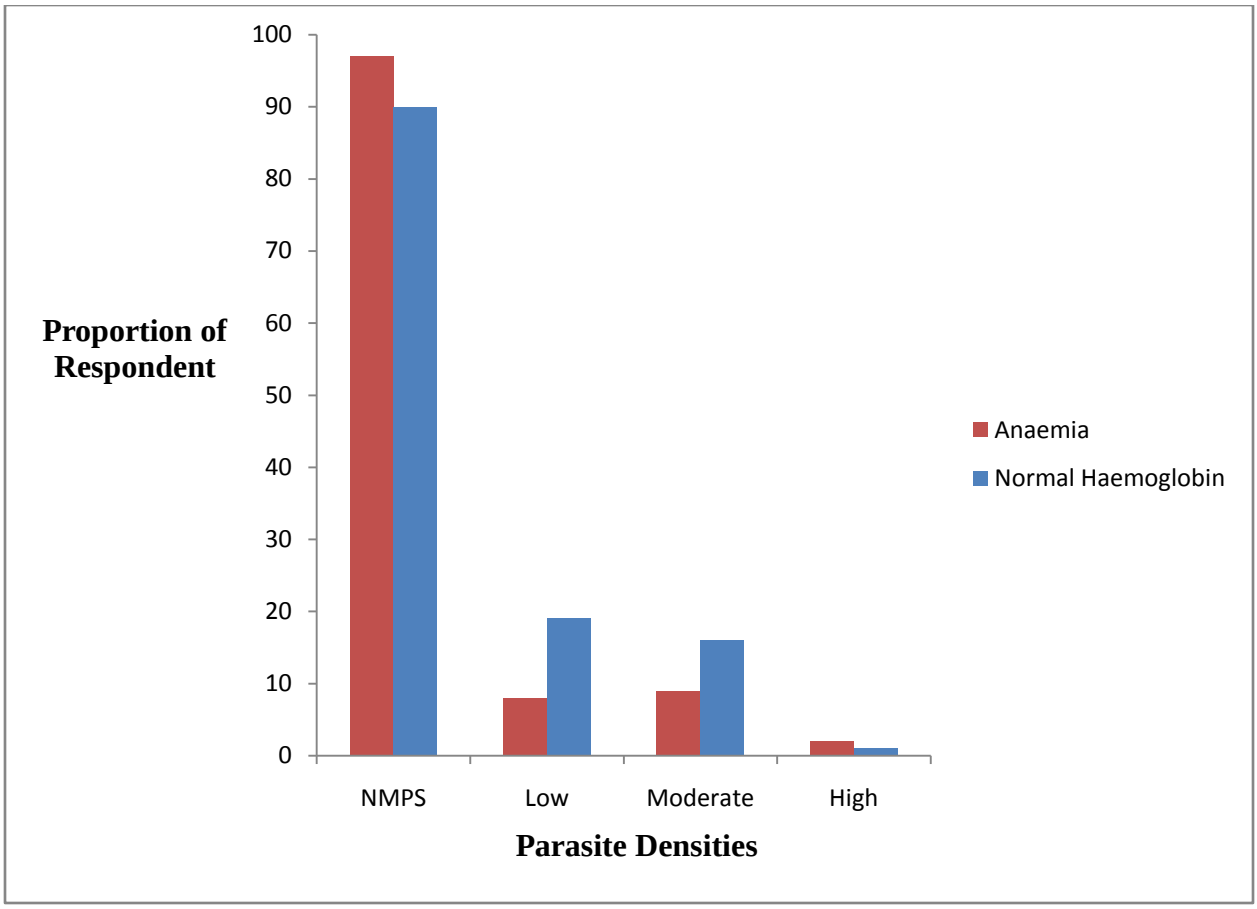

Figure 4.2: Haemoglobin level in relation to parasites density by women on antenatal care in General Hospital Nassarawa-Eggon, Nasarawa State

Most of the women (83.6%) with no malaria parasitaemia had normal haemoglobin level, while anaemia was found to be more (15. 1%) and (12.7%) amongst women with low and moderate parasites density respectively. However, some few women with high parasitaemia (1.7%), had normal haemoglobin level (≥11g/dl) (Fig 4.2).

Table 4.5: Multivariate analysis of factors associated with malaria parasitaemia among women on ANC in General Hospital, Nassarawa-Eggon, Nasarawa State (n=242)

Factors	AOR	95% CI	P-value
Age < 25	0. 4	0. 2 – 0. 8	0. 01
Non-usage of LLIN	0. 4	0. 2 – 0. 9	0. 02
Primigravidae	0. 8	0. 4 – 1. 5	0. 45
Haemoglobin Level	1. 9	1. 0 – 3. 5	0. 05

Age < 25years and non-usage of LLIN were independently statistically significant on logistic regression while gravidity and anaemia were not (Table 4.5).

CHAPTER FIVE

DISCUSSION

The prevalence of asymptomatic malaria parasitaemia in this study was 25. 6% by RDT screening and 22.7% by microscopy. This difference could be due to possible placental sequestration of parasites[18] and the persistence of histidine rich protein 2 (HRP-2) antigen of *P. falciparum* even after the parasite clearance.[87] The RDT used had a sensitivity of 98% and a specificity of 97. 5% (as contained in the manufacturers Insert) compared to conventional microscopy which is considered as the gold standard.[88] These results are similar to those from studies done in Ouagadougou, Burkina Fasoon the prevalence of asymptomatic malaria among pregnant women and found a prevalence of 30% and 24% by RDT and microscopy respectively.[23] It is also comparable with a study done in Ondo South district, Nigeria on the prevalence of asymptomatic malaria parasitaemia in pregnant unbooked women with a prevalence of 25. 9% using RDT[21] and a study in Lagos, Nigeria which accessed knowledge, attitude, perception and home management of malaria among consenting pregnant women attending antenatal care (ANC) clinic with a prevalence of 27. 4% by microscopy.[89]

The only species of malaria parasite identified in this study was *Plasmodium falciparum*. This is similar to findings of studies done in Libreville, Gabon[64]which looked at the relationships between malarial parasitaemia and age, gravidity and anaemia in pregnant women and Minna, Nigeria[84]which looked at the true prevalence of congenital, cord, and placental malaria in which only *Plasmodium falciparum* was identified. It is also in agreement with the widely accepted view that *Plasmodium falciparum* is the predominant species in Nigeria accounting for about 98% of malaria cases in the country.[64,90]

Age<25years and Primigravidae were significantly associated with malaria parasitaemia and parasite density in this study. This means that a pregnant woman of a younger age and Primigravidae are at a greater risk of malaria parasitaemia than those who have been pregnant several times and have acquired specific immunity to placental malaria due to previous exposure[18,44] which increases with subsequent infection and pregnancies.[91]Findings have been reported in Libreville, Gabon on the relationships between malarial parasitaemia and age, gravidity and anaemia in pregnant women and found that Primigravidae and young pregnant women were the most susceptible to infection.[87]This could also be due to hormonal and immunological changes which lower their immunity in pregnancy. The significant association found in this study is similar to the findings of a study done in Mozambique where the clinical presentation of malaria in African women was characterized.[93]

The use of LLIN is one of the preventive strategies of malaria in pregnancy. It has been reported that the use of LLIN substantially reduces the risk of malaria in pregnancy.[94] For this study, the use of LLIN was protective for the presence of malaria parasitaemia. However the use of LLIN was significantly associated with parasites density. This means that while LLIN can reduce the risk of being exposed to malaria parasitaemia, it has no effect on the multiplication of the parasites once in the system. The outcome of this finding is in line with the fact that LLIN substantially reduce the risk of malaria in pregnancy when effectively used. However, the effective use of LLIN has its own challenges; such that out of the 24hours of the day, a pregnant women can be under the net for a maximum of 8-10hrs, and some may have the net but may not be sleeping under it always, while some may have complain of heat and related excuses, and the net may even have holes thus creating a number of chances for being bitten or getting infected with malaria. Once infected, LLIN has no effect on the multiplication of the parasites in the

system. The first outcome is consistent with previous studies in Okpoko area of Anambra State, Southeast Nigeria on the effects of the use of LLIN on episodes of uncomplicated malaria, frequencies of malaria parasitaemia and anaemia among primigravidae in which LLIN was shown to reduce the number of infective mosquito bites by 70. 0 –90.0% in a variety of ecologic settings.[95]

This study also indicated a significant association between anaemia, the presence of parasitaemia and parasites density. This is understandable as malaria parasitaemia results in the destruction of red blood cells and particularly for *P. falciparum* which affects both young and new red blood cells and consequently a potential cause ofanaemia.[92]This is in line with the fact that malaria is an under-recognized cause of anaemia in pregnancy in endemic areas like ours and is usually asymptomatic.[71]

From the logistic regression done in this study, we did not find any statistically significant association of malaria parasitaemia with gravidity and haemoglobin level. This is in line with findings in a study done in Calabar, Nigeria on *P. falciparum* asymptomatic malaria in pregnant women, in which there was no statistically significant difference in the prevalence of anaemia among the Primigravidae and the multigravidae.[15]However, age 25years and lower and non-usage of LLIN were statistically significant on logistic regression. This means that younger maternal age in pregnancy are more susceptible to malaria parasitaemia. This is similar to a previous report on the significance of malaria parasitaemia with regards to maternal age in pregnancy in Yaounde Cameroun[52] and also supports the importance of the use of LLIN as a preventive strategy. This is also in agreement with a previous report on the systematic review of insecticide treated net for the prevention of malaria in pregnancy.[94]

The relationship between haemoglobin level and parasites density as indicated in figure 2 shows the impact of malaria parasitaemia on the haemoglobin level of the pregnant women. Consequently, in the absence of parasitaemia, more women had a normal level of haemoglobin, but with low and moderate levels of parasitaemia, more of the women had anaemia. However, a few of the women had high parasitaemia, yet normal haemoglobin level, this indicates the very few in endemic areas who with continuous exposure had developed some level of immunity. The outcome of this study is similar to a previous study in Calabar, Nigeria which looked at the prevalence of anaemia in asymptomatic malaria parasitemic women at first antenatal visit in a tertiary hospital facility.[14]

The prevalence of malaria parasitaemia as identified in this study by employing two methods [Microscopy (the gold standard), and RDT screening], indicates a satisfactory level of effectiveness in the use of malaria RDT for diagnostic purpose in our health facility. A similar report was made from a study on the diagnostic performance of histidine-rich protein 2 (HRP-2) based malaria RDT kit in Kaduna, Nigeria.[96]

CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6. 1 Conclusion

The level of asymptomatic malaria parasitaemia revealed in this study is reasonably high. Younger age of less than 25 years had highest risk of malaria parasitaemia. Failure to use LLIN is associated with an increased risk of malaria infection. Malaria parasitaemia can be responsible for anaemia in pregnant women and may be responsible for mother to child transmission of malaria. Occupation and gravidity can also be associated with malaria parasitaemia and the degree of anaemia can be related to the parasites density. The performance of RDT for malaria screening in this study is comparable with Microscopy as the Gold Standard for use in our health facilities.

6. 2 Recommendation

Asymptomatic malaria parasitaemia is high among the study population. There is a need to educate the people, especially the women on preventive measures against malaria.

There is a need to take campaign programmes on malaria to schools targeting younger age group.

For more effective malaria prevention, there is a need to introduce routine diagnosis of malaria infection as part of the antenatal care strategy in Nigeria.

The ministry of health should intensify campaign on the potential effects of malaria in pregnancy and the need for a more effective use of LLIN. The administration of IPT should also be intensified.

REFERENCES

1. Conroy A, McDonald C, Kain K. Malaria in pregnancy: diagnosing infection and identifying fetal risk; Expert Review. Anti Infect Ther. 2012;10(11):1331–42.
2. WHO_Malaria. WHO _ Malaria, Immunization, Vaccines and Biologicals. [Internet]. 2013. Available from: http://www.who.int/immunization/topics/malaria/en/
3. WHO. Factsheet on the World Malaria Report, [Internet]. 2014. Available from: http://www.who.int/malaria/media/world_malaria_report_2014/en/
4. Malaria RB. Federal Ministry of Health. National malaria control programme in Nigeria Abuja. 2005.
5. Schantz-Dunn J, Nawal MN. Malaria and Pregnancy A Global Health Perspective. Rev Obs Gynecol [Internet]. 2009;2(3):186–92. Available from: http://apps.who.int/malaria/pregnantwomenandinfants.html.
6. Wickaramsuriya G. Clinical Features of Malaria in Pregnancy. Malaria and Ankylostomiasis in Pregnant Women. Oxford University Press, London.; 1937.
7. WHO. World malaria report [Internet]. 2008. Available from: www.malaria.org/malaria2008.pdf
8. Costa F, Avril M, Nogueira P, Gysin J. Cytoadhesion of Plasmodium falciparum-infected erythrocytes and the infected placenta: a two-way pathway. Brazilian J Med Biol Res. 2006;39:1525–36.
9. Ishag A, Amar H, Mustafa I. Prevalence and risk factors for Plasmodium falciparum malaria in pregnant women of eastern Sudan. Malar J [Internet]. 2005;4(18):1475–2875. Available from: http://www.malariajournal.com/content/4/1/18
10. Ogbodo SO, Nwagha UI, Okaka ANC, Ogenyi SC, Okoko RO, Nwagha TU. Malaria Parasitaemia Among Pregnant Women in a Rural Community of Eastern Nigeria;need for combined measures. Niger J Physiol Sci. 2009;24(2):95–100.
11. Arulogun OS, Okereke CC. Knowledge and practices of intermittent preventive treatment of malaria in pregnancy among health workers in a southwest local government area of Nigeria. J Med Med Sci [Internet]. 2012;3(6):415–22. Available from: http://www.interesjournals.org/JMMS
12. Njama-Meya D, Kamya MR, Dorsey G. Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Ugandan children. Trop Med Int Heal [Internet]. 2004;9(8):862–8. Available from: http://onlinelibrary.wiley.com/doi/10.1111/tmi.2004.9.issue-8/issuetoc
13. Shulman C, Dorman E. Importance and prevention for malaria in pregnancy. Trans R Soc Trop Med Hyg. 2003;97:30–5.
14. Agomo CO, Oyibo WA. Factors associated with risk of malaria infection among pregnant women in Lagos , Nigeria. Infect Dis poverty [Internet]. 2013;2(1):1. Available from: http://www.idpjournal.com/content/2/1/19
15. Agan T, Ekabua J, Udoh A, Ekanem E, Efiok E, Mgbekem M. Prevalence of anemia in women with asymptomatic malaria parasitemia at first antenatal care visit at the University of Calabar. Int J Womens Health. 2010;2:229–33.

16. WHO, Malaria Report. Factsheet on the World Malaria Report 2013. 2013; 1–3.
17. WHO RMP. Rolling Back Malaria in member states of The Organisation of Islamic Cooperation – Events archive 2007-2013 [Internet]. 2014. Available from: http://www.rbm.who.int/docs/2014/RBM-OIC-Booklet.pdf
18. Elliott SR, Brennan AK, Beeson JG, Tadesse E, Molyneux ME, Brown GV et al. Placental malaria induces variant antibody of the cytophylic subtype immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity. Infect Immun. 2005;73:5903–7.
19. Chigozie J. Uneke. Impact of Placental Plasmodium falciparum Malaria on Pregnancy and Perinatal Outcome in Sub-Saharan Africa. Yale J Biol Med. 2007;80(2):39–50.
20. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B NR. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93–104.
21. Nwaneri DU, Adeleye OA, Ande AB. Asymptomatic malaria parasitaemia using rapid diagnostic test in unbooked pregnant women in rural Ondo-south district , Nigeria. J Prev Med Hyg. 2013;54:49–52.
22. WHO. World Health Organisation. Roll Back Malaria: Malaria in pregnancy [Internet]. RBM infosheet. 2007. Available from: http://www.rbm.who.int/cmc_upload/0/000/015/369/RBMInfosheet_4.htm
23. Douamba Z, Bisseye C, Djigma FW, R T, Compaoré, Valérie Jean Telesphore Bazie VP, Nikiema J-B, et al. Asymptomatic Malaria in Pregnancy. J Biomed Biotechnol [Internet]. 2012;198317. Available from: http://www.ncbi.nlm.nih.gov/pubmed/?term=Douamba Z%5Bauth%5D
24. WHO. Lives at Risk: Malaria in pregnancy. 2003; Published 2003. WHO Inf sheet [Internet]. 2003; Available from: http://www.who.int/features/2003/04b/en/
25. Okpere E. Malaria in pregnancy. Clin Obstet. 2004;56–61.
26. Steketee R, Nahlen B, Parise M, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. Am J Trop Med Hyg. 2001;64:28–35.
27. Collins W. Plasmodium knowlesi: A malaria parasite of monkeys and humans. Annu Rev Entomol. 2012;57:107–21.
28. Nadjm B, Behrens R. Malaria: An update for physicians. Infect Dis Clin North Am. 2012;26(2):243–59.
29. Collins W, Barnwell J. Plasmodium knowlesi: finally being recognized. J Infect Dis. 2009;199(8):1107–8.
30. Wadie BO. Molecular approach to malaria. Med Parasitol. 2002;28:1671–80.
31. Chloe B, Laurence-knott. Malaria in Pregnancy-online [Internet]. online. 2013 [cited 2015 Mar 21]. Available from: http://www.patient.co.uk/authors/dr-laurence-knott
32. CDC. The Rapid Assessment of the Burden of Malaria during Pregnancy: A Toolkit. 2010.
33. Takem E, D'Alessandro U. Malaria in pregnancy. Mediterr J Hematol Infect Dis. 2013;5(1):e2013010.
34. Adam I, Khamis AH, Elbashir MI. Prevalence and risk factors for Plasmodium falciparum

malaria in pregnant women of eastern Sudan. Malar J. 2005;102(2003):3–7.

35. World Health Organization. World Health Statistics. 2012.
36. Rijken M, McGready R, Boel M et al. Malaria in pregnancy in the Asia-Pacific region. Lancet Infect Dis. 2012;12(1):75–88.
37. The Global Fund WS. Malaria. [Internet]. 2009. Available from: www.theglobalfund.orgocuments/publications/diseasereport/
38. Riley E, Hviid L, Theander T. Malaria. In Parasitic Infections and the Immune System. Kierszenbaum F., editor. New York: Academic Press; 1994.
39. Moudou C, Ibrahim S, Guekoun L, Sanata B, Dramane B, Robert TG. Prevalence and risk factors for Plasmodium falciparum malaria in pregnant women attending antenatal clinic in Bobo-Dioulasso (Burkina Faso). BMC Infect Dis [Internet]. 2014;14:631. Available from: http://www.biomedcentral.com/1471-2334/14/631
40. Monif G, Baker D. Infectious Disease in Obstetrics and Gynecology. In: 6th ed. New York: Parthenon. 2004. 280–286 p.
41. WHO. WHO, (2006). Guidelines for the Treatment of Malaria. Geneva. 2006.
42. GAW W. Some observations on malaria occurring in association with pregnancy. J Obstet Gynaecol (Lahore). 1935;42:816–34.
43. Mwanziva C, Shekalaghe S, Ndaro A, Mengerink B, Megiroo S, Mosha F, et al. Overuse of artemisinincombination therapy in Mtowa Mbu (river of mosquitoes), an area misinterpreted as high endemic for malaria. Malar J. 2008;7:232.
44. Staalsoe T, Shulman C, Buhner J, Kawuondo K, Marsh K, Hviid L. Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated Plasmodium falciparum malaria. Lancet. 2004;363:283–9.
45. Rogerson S, Boeuf P. New approaches to malaria in pregnancy. Parasitology. 2007;134:1883–93.
46. Philippe B, Aitken EH, Chandrasiri U, Chua CLL, McInerney B, McQuade L, et al. Plasmodium falciparum Malaria Elicits Inflammatory Responses that Dysregulate Placental Amino Acid Transport. DOI: 10.1371/journal.ppat.1003153. 2013.
47. Enato E, Okhamafe A, Okpere E, Oseji F. Prevalence of Malaria during Pregnancy and Antimalarial Intervention in an Urban Secondary Health Care Facility in Southern Nigeria. Med Princ Pr. 2007;16(3):240–3.
48. Nahlen BL. Rolling back malaria in pregnancy. N Engl J Med. 2000;343:651–2.
49. Diagne N, Rogier C, Sokhna S. Increased susceptibility to malaria during the early postpartum period. N Engl J Med. 2000;343:598–605.
50. Rogerson SJ, Hviid L, Duffy P, Leke R, Taylor D. Malaria in pregnancy: pathogenesis and immunity. Lancet Infect Dis. 2007;7(2):105–17.
51. Brabin BJ, Romagosa C, Abdelgalil S. The sick placenta-the role of malaria. Placenta. 2004;25:359–78.
52. Tako EA, Zhou A, Lohoue A, Leke R, Taylor DW, Leke RF. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde Cameroun. Am J Trop Med Hyg.

2005;236–42.

53. Shulman CE, Marshal M, Dorman EK. Malaria in pregnancy: adverse effects on hemoglobin level and birth weight in primigravidae and multigravidae. Trop Med Int Heal. 2001;6:770–8.

54. Ducarme G, Thuillier C, Wernet A, Bellier C, Luton D. Malaria in pregnant woman masquerading as HELLP syndrome. Am J Perinatol [Internet]. 2010;27(2):171–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19626579

55. Okpere EE. Malaria in pregnancy. In Okpere E (Ed): Clinical Obstetrics. Uniben Press; 2004. 56-63 p.

56. Bouyou-Akotet, M. K Adegnika AA, Agnandji ST, Ngou-Milama E, Kombila M, Kremsner PG et al. Cortisol and susceptibility to malaria during pregnancy. Microbes Infect. 2005;7(11-12):1217–23.

57. Lindsay S, Ansell J, Selman C, Cox V, Hamilton K, Walraven G. Effect of pregnancy on exposure to malaria mosquitoes. Lancet. 2000;355(9219):1972–5.

58. Martvnez-Espinosa, FE Alecrim W, Daniel-Ribeiro C. Attraction of mosquitoes to pregnant women. Lancet. 2000;356(9230):685.

59. Enato EF, Mens PF, Okhamafe AO, Okpere EE, Pogoson E, Shalling HD. Plasmodium falciparum malaria in pregnancy: prevalence of peripheral parasitemia, anaemia and malaria care seeking behavior among pregnant women attending 2 antenatal clinics in Edo state, Nigeria. J Obstet Gynaecol. 2009;29(4):301–6.

60. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. Nature. 1998;395:851–2.

61. Duffy PE, Fried M. Malaria during pregnancy; parasites, antibodies and chondroitin sulphate- A. Biochem Soc Trans. 1999;27:478–82.

62. Rasheed FN, Bulmer JN, Dunn DT, Menendex C, Jawla MFB, Jepson A et, et al. Suppressed peripheral blood and placental blood lymphoproliferative responses in first pregnancies: relevance to malaria. Am J Trop Med Hyg. 1993;48:154–60.

63. Riley EM, Schneider G, Sambou I, Greenwood BM. Suppression of cell mediated immune responses to malaria antigens in pregnant Gambian women. Am J Trop Med Hyg. 1989;40:141–4.

64. Bouyou-Akotet, M. K Lonete-Collard DE, Mabika-Manfoumbi, M Kendjo E, Matsiegui, P. B Mavoungou E et al. Prevalence of Plasmodium falciparum infection in pregnant women in Gabon. Malar J. 2003;2:18.

65. Achidi E, Kuoh A, Minang J, Ngum B, Achimbom B, Motaze S, et al. Malaria infection in pregnancy and its effects on haemoglobin levels in women from a malaria endemic area of Fako Division, South West Province, Cameroon. J Obstet Gynaecol [Internet]. 2005;25(3):235–40. Available from: http://informahealthcare.com

66. Omigbodun AO. Recent trends in the management of anaemia in pregnancy. Trop J Obstet Gynaecol. 2004;21(1):1–3.

67. Komolafe J, Kuti O, Oni O, Egbewale B. Sociodemographic characteristics of anaemic gravidae at booking: A preliminary study at Ilesha, western Nigeria. Niger J Med.

2005;14(2):151–4.

68. Harrison K. Anaemia in pregnancy. In: Lawson JB, Harrison KA, Bergstrom S, editors. Maternity care in developing countries. RCOG Press. 2001. 112–128 p.

69. Ogunbode O. Anaemia in pregnancy. In: Okonofua F, Odunsi K, editors. Contemporary obstetrics and gynaecology for developing countries. WHARC. 2003. 514–529 p.

70. Health FM of H. Federal Ministry of Health. Nigerian. National Antimalarial Treatment Guidelines. 2005 p. 1–29.

71. Agan T, Ekabua J, Iklaki C, Oyo-Ita A, Ibanga I. Prevalence of asymptomatic malaria parasitaemia. Asian pacific J Trop Med. 2010;3(1):51–4.

72. Hisano M, Suzuki R, Sago H, Murashima A, Yamaguchi K. Vit B6 deficiency and anaemia in pregnancy. Eur J Clininical Nutr. 2010;64:221–3.

73. Aimakhu C, Olayemi O. Maternal haematocrit and pregnancy outcome in Nigerian women. West Africa J Med. 2003;22(1):18–21.

74. Gwer S, Newton C, Berkley J. Overdiagnosis and co-morbidities of severe malaria in African children: a guide for clinicians. American. J Trop Med Hyg. 2007;77(6):6–13.

75. Hussain N, Echoga A, Iwarere O. Pattern of malaria presentation and treatment at Obiesan Naval Medical Centre, Lagos- Nigeria. Eur J Sci Res. 2009;27(1):120–7.

76. Berrang –Ford L, MacLean J, Gyorkos T, Ford J, Ogden N. Climate change and Malaria in Canada: a systems approach. Interdiscip Perspect Infect Dis. 2008;2009.

77. Orogade A, Falade C, Okafor H, Mokuolu O, Mamman A, Tagbo A, et al. Clinical and laboratory features of congenital malaria in Nigeria. J Pediatr Infect Dis. 2008;3:181–7.

78. Keiser J, Utzinger J, Premji Z, Yamagata Y, Singer B. Acridine Orange for malaria, the Diagnosis: its diagnostic performance; its promotion and implementation in Tanzania and implications for malaria control. Ann Trop Med Parsitology. 2002;97(6):643–54.

79. Myjak P, Nahorski W, Pieniazek N, Pietkiewicz H. Usefulness of PCR for Diagnosis of Imported Malaria in Poland. Eur J Clin Microbiol Infect Dis. 2002;21:215–8.

80. World Health Organization. World Malaria Report [Internet]. 2010. Available from: www.who.int/malaria/world_malaria_report_2010p://

81. Meeusen E, Bischof R, Lee C. Comparative T-cell responses during pregnancy in large animals and humans. Am J Reprod Immunol. 2001;46:169–79.

82. Kalanda G, Hill J, Verhoeff F, Brabin B. Comparative efficacy of chloroquine and sulfadoxine-pyrimethamine in pregnant women and children: a metaanalysis. Trop Med Int Heal. 2006;11:569–77.

83. Ayiwulu E. The nature of water scarcity and domestic water demand in Nassarawa Eggon town of Nasarawa state. Dissertation, University of Jos. 2012.

84. Chukwuemeka Innocent James O, Charles M, Amaka M, Ayanwale V, Olayemi IK, Lateef A, et al. Prevalence of Congenital Malaria in Minna, North Central Nigeria. J Trop Med. 2012;2012:274142.

85. Antwi GD. Factors influencing the uptake of intermittent preventive treatment of malaria in pregnancy in the Bosomtwe district of Ghana. 2010.

86. Jain NC. Schalm's Veterinary Hematology. 4th ed. Lea & Febiger.Wiley-Black well, Philadelphia.; 1986. 1221 p.

87. Kattenberg J, Tahita C, Versteeg I et al. Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso and the implications for the diagnosis of malaria in pregnancy. Trop Med Int Heal. 2012;17(2):550–7.

88. WHO. World malaria report 2008. Geneva, Switzerland: World Health OrganizationPress [Internet]. 2008. Available from: www.malaria.org/malaria2008.pdf

89. Iriemenam N, Dosunmu A, Oyibo W et al. Knowledge, attitude, perception of malaria and evaluation of malaria parasitaemia among pregnant women attending antenatal care clinic in metropolitan Lagos, Nigeria. J Vector Borne Dis. 2011;48:12–7.

90. Federal Ministry of Health (FMOH). National diagnosis and treatment policy. Abuja – Nigeria: Federal Ministry of Health Nigeria, National Malaria and Vector Control Division. 2010.

91. TerKuile F, Terlouw D, Phillips-Howard P, Hawley W, Friedman J, Kariuki S, et al. Reduction of malaria during pregnancy by permethrin-treated bed nets in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg [Internet]. 2003;64(2):50–60. Available from: www.ncbi.nlm.nih.gov

92. Marielle K, Denisa E, Modeste M, Eric K, Pierre B, Elie M, et al. Prevalence of Plasmodium falciparum infection in pregnant women in Gabon. Malar J. 2003;2:18.

93. Bardají A, Sigauque B, Bruni L, Romagosa C, Sanz S, Mabunda S et al. Clinical malaria in African pregnant women. Malar J [Internet]. 2008;7:27. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2267805

94. Gamble C, Ekwaru P, Garner P, ter Kuile F. Insecticide-treated nets for the prevention of malaria in pregnancy: A systematic review of randomized controlled trials. PLoS Med [Internet]. 2007;4(3):e107. Available from: http://journals.plos.org/plosmedicine/article/asset?id=10.1371/journal.pmed.0040107.XML

95. Igwe PC, Inem V, Ebuehi OM, Afolabi BM. The effect of insecticide treated bed net use on malaria episodes , parasitaemia and haemoglobin concentration among primigravidae in a peri-urban settlement in southeast Nigeria. J Rural Trop Public Heal [Internet]. 2007;6:25–32. Available from: http://www.jcu.edu.au/jrtph/vol/v06ebuehi.pdf

96. Olufemi Ajumobi, Kabir Sabitu, Patrick Nguku, Jacob Kwaga, Godwin Ntadom, Sheba N. Gitta, Elizeus Rutebemberwa, Wellington Oyibo, Peter Nsubuga, Mark Maire GP. Performance of an HRP-2 Rapid Diagnostic Test in Nigerian Children Less Than 5 Years of Age. Am J Trop Med Hyg. 2015;94(4).

APPENDIX 1

ETHICAL CLEARANCE

NASARAWA STATE OF NIGERIA

MINISTRY OF HEALTH

In repling, please quote reference and date
All Correspondence should be directed to
the Commissioner

Ministry of Health Headquarters
Private Mail Bag 032
Lafia, Nasarawa State.

Telephone:

S/MOH/843/

26th May, 2014

Emiasegen Samuel Ebuga,
Department of Community Medicine,
Ahmadu Bello University,
Zaria.

RE: APPLICATION FOR ETHICAL CLEARANCE

Reference to your letter of 5th May,2014 to conduct the study titled " Malaria Parasitaemia and Associated Risk Factors Among Pregnant Women Attending Antenatal Clinic in General Hospital N/Eggon, Nasarawa State, Nigeria", I am directed to convey the Ministry's approval for the conduct of the study.

2 Accordingly, you are to strictly adhere to the methodology in your proposal and to also seek permission from the head of the health facility that you will use for the study.

3 At the end of the study, you must forward two copies of the report to the ministry for our record, please.

Dr Ekom G. Haruna.
Ag .Director Clinical Services,
for: Honourable Commissioner

APPENDIX 2

CONSENT FORM

I wish to indicate my interest to participate in this research work titled; FACTORS ASSOCIATED WITH ASYMPTOMATIC MALARIA PARASITAEMIA AMONG WOMEN ATTENDING ANTENATAL CLINIC IN NASSARAWA EGGON GENERAL HOSPITAL, NASARAWA STATE.

I have been made to understand the purpose of the research and my requirements as a participant which includes answering some questions in the questionnaire and collection of 3mls of blood from the cubital fossa in the arm of my hand.

I hereby consent to participate in the research out of my free will, and for the benefit of public health.

Name ……. .
Phone number ……. . . .
Signature/Date ……

APPENDIX 3

QUESTIONNAIRE

Topic: This is a study to determine the Factors Associated with Asymptomatic Malaria Parasitaemia among women attending Antenatal Clinic in Nassarawa-Eggon General Hospital, Nasarawa State. The result will be used to guide government policy for the benefit of the people and confidentiality of information will be maintained.

Your participation will help to identify some of the problems associated with asymptomatic malaria parasitaemia in your area and your participation is voluntary.

If you are participating, a few minute of your time will be required to fill this form, and a little discomfort from needle stick for blood sample to be collected.

Date: _____/_____/_____ Interviewer's Name:_____

Day/Month/Year

Questionnaire number: ___ ___ ___

Have you been administered similar questionnaire in the last three months? Yes [], No []

DEMOGRAPHIC CHARACTERISTICS

1. **Age …….**
2. **Marital status ……. .**

[a] Married or living together, [b] Divorced / Separated, [c] Widowed, [d] Single

3. **What is the highest level of education you have attained?**

[a] None, [b] Primary, [c] Secondary, [d] Post-Secondary

4. **What is your main occupation?**

[a] House wife, [b] Civil Servant, [c] Business, [d] Student

I. MALARIA AS A DISEASE

5. **Have you ever heard of malaria?** [a] Yes, [b] No, [c] Don't know
6. **How does someone acquire malaria?** (Do not read answers, circle all that apply)

[a] Mosquitoes bite, [b] Unclean environment, [c] Don't know

II. MALARIA PREVENTION

7. What can you use as intermittent preventive treatment for malaria during pregnancy?

[a] Use a mosquito net, [b] Spray insecticides inside, [c] Burn local plants/herbs

[d] Use SP [e] Don't know

8. Do you have or use long lasting insecticide net (LLIN) [1] Yes, [2] No

9. How did you get your LLIN?

[a] Free from the government source, [b] Through a voucher scheme, [c] Bought ourselves

III. ANTENATAL CARE

10. How many children have you?

[a] 1, [b] 2, [c] More than 3

11. Have you attended ANC before now during this pregnancy?

[1] Yes) [2] No

12. How old is your pregnancy?

[a] 1-3 months, [b] 4-6 months, [c] 7-9 months

13. Which of the months did you attend your first ANC?

[a] 1-3 months, [b] 4-6 months, [c] 7-9 months

14. During this pregnancy, have you suffered/ been treated for malaria?

[1] YES, [2] NO

15. Did you receive a blood transfusion during this pregnancy?

[1] Yes, [2] No

16. Did you receive information on malaria prevention and treatment at the ANC?

[1] YES, [2] NO

17. What is the drug of choice for malaria prevention during pregnancy?

[a] SP, [b] Other, specify……, [c] Don't know

18. During ANC visit in your last pregnancy did the nurse give you SP to swallow while observing you?

[1] Yes, [2] No

19 Any history of miscarriage/abortion?

[1] Yes, [2] No

LABORATORY EXAMINATIONS RESULTS

Hemoglobin level (g/dl): …….

RDT result: Positive [ ], Negative [ ]

Blood film result ……. .

Malaria species. Check all that apply.

Plasmodium falciparum [ ]

P. vivax [ ]

P. malariae [ ]

P. ovale [ ]

Parasite density per ul……. .

APPENDIX 4

RDT INSERT 1

Rev. (2014)

CareStart™ Malaria HRP2 (Pf)

A rapid test for the detection of malaria HRP2 (histidine-rich protein 2) in human blood

Intended Use

For the rapid qualitative detection of malaria HRP2 (histidine-rich protein 2) of *P. falciparum*) in human blood as an aid in the diagnosis of malaria infection.

Summary

Malaria is a serious parasitic illness characterized by fever, chills, and anemia and is caused by a protozoan parasite that is transmitted to humans through bites of infected *Anopheles* mosquitoes. There are four types of human malaria: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. In humans after an infected mosquito bites, the parasites (called sporozoites) migrate to the liver where they mature and are released into blood stream, infecting red blood cells. Malaria infection occurs in more than 90 countries worldwide, but is mostly prevalent in sub-Saharan Africa. It is estimated that there are over 500 million clinical cases and 2.7 million malaria caused deaths per year.

The CareStart™ Malaria HRP2 (Pf) contains a membrane strip, which is pre-coated with a monoclonal antibody as single line across a test strip. The monoclonal antibody is specific to the HRP2 (histidine-rich protein 2) of the *P. falciparum*. The conjugate pad is dispersed with antibody absorbed on gold particles which are specific to HRP2 of *P. falciparum*.

The CareStart™ Malaria HRP2 (Pf) is designed for the diagnosis of *P. falciparum* infection.

Materials Provided

CareStart™ Malaria HRP2 (Pf) contains the following items:

Test Device (Test device sealed in aluminum pouch with desiccant)

Instructions for Use

IFU-CO181-MPF-Rev.A

Assay Buffer (Borax buffered SDS and saponin solution)

Optional: sample pipette / lancet / alcohol pad

- Sample pipette description and instruction -

1) Gently squeeze the pipette.
2) Touch the blood with the open end of the pipette.
3) Gently release to aspirate blood up to the black mark.

Warnings and Precautions

The following requirements must be observed:

1) For in vitro diagnostic and professional use only.
2) Read provided instructions for use before using the test kit.
3) Observe storage condition indicated on the aluminum pouch and outer package.
4) Do not use components from other lots.
5) Use the test device as it comes. components (lancet / alcohol pad) immediately after opening its package.
6) Use disposable protective gloves while handling potentially infectious materials and perform only the assay. Wash hands thoroughly afterwards.
7) Do not swallow the Assay Buffer solution.
8) Do not use the kit beyond the expiration date that is indicated on the outer package.
9) All provided materials are single-use. do not re-use any of contents.
10) Do not eat or smoke while handling specimens.
11) Clean up spills thoroughly using an appropriate disinfectant.
12) The test procedure, precautions, and interpretation of results for this assay must be closely followed when testing.
13) If the test kit is stored in a refrigerator (Do not freeze the test kit), the devices need to equilibrate to room temperature prior to use.
14) Do not use, if lancet cap is open and/or if package of alcohol pad or device pouch is damaged.

Access Bio, Inc.

Test Procedure

1) Clean the area to be pierced with an alcohol pad.
2) Squeeze the end of a fingertip and pierce it using venipuncture or provided lancet.
3) Wipe out the first drop of blood with sterile gauze or cotton. (Not provided.)
4) Collect the blood sample (5 µl) using pipette provided or micro-pipette.
5) Add whole blood (5 µl) to the sample well.
6) Add assay buffer 2 drops (80 µl) to the buffer well.
7) Read result in 20 minutes.

Interpretation of the Test Result

1) Invalid
The test is invalid if the line in the "C" area does not appear. If this occurs, the test should be repeated using new device.

2) Negative
The presence of only one line in the "C" area indicates negative result.

3) Positive
The presence of two color bands (one band in the "C" and another band in the "T") indicates a positive result for *P. falciparum*.

CareStart™ Malaria (Product Code)

APPENDIX 5

RDT INSERT 2

Limitations and Interferences

1) Anti-coagulants such as heparin, EDTA, and citrate do not affect the test result.
2) This test is designed to detect of antigens of Malaria *Plasmodium* sp. Although the test is highly reliable in detecting HRP2, false result may be possible in rare occasions. Therefore, other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of this test, but should only be made by a qualified physician after all clinical and laboratory findings have been evaluated.
3) If specimens cannot be immediately tested, they should be refrigerated at 2 – 8°C up to 3 days. For storage periods longer than three days, freezing is recommended.

Performance Characteristics

The *CareStart*™ Malaria HRP2 (Pf) has been tested with positive and negative clinical samples conformed by microscopic examination. The results are shown tables below;

1) Results of *P. falciparum* evaluation

Contents	P.f positive confirmed specimen		Sensitivity
	Positive	Negative	
CareStart™ Malaria HRP2 (Pf)	98	2	98%

2) Results of negative human blood specimen evaluation

Contents	Random normal human specimen		Specificity
	Positive	Negative	
CareStart™ Malaria HRP2 (Pf)	5	195	97.5%

Precision

Within- and between-run precisions have been evaluated by the testing 10 replicates of three specimens: a negative, a low positive and a strong positive. The agreement between the test results and the expected results were 100%.

References

1) Valecha N., Eapen A., Devi C. Usha, Ravindran J., Aggarwal A., and Subbarao S. K. (2002). Field evaluation of the ICT Malaria P.f/P.v. immunochromatographic test in India. Annals of Tropical Medicine & Parasitology. 96: 333-336
2) Iqbal J., Hira P. R., Sher A., and AL-Enezi A. A. 2001. Diagnosis of imported Malaria by Plasmodium Lactate Dehydrogenase (pLDH) and Histidine-Rich Protein 2 (PfHRP2)-based immunocapture assays. American Journal of Tropical Medicine and Hygiene. 64: 20-23
3) Tjitra E., Suprianto S., Dyer M., Currie B. J. and Anstey N. M. (1999). Detection of Histidine-rich Protein 2 and panmalarial ICT MALARIA P.f/P.v. test antigens after chloroquine treatment of uncomplicated falciparum malaria does not reliably predict treatment outcome in eastern Indonesia. Journal of Clinical microbiology. 37: 2412-2417
Panton L. J., PePhie P., Maloy W. L., Wellems T. E., Taylor D. W. and Howard R. J. (1989). Purification and partial characterization of an unusual protein of Plasmodium falciparum: histidine-rich protein II. Molecular

Description of Symbol Used

	Single use		Authorized representative in EU
	Use by		Sufficient for
LOT	Batch code	IVD	In-Vitro diagnostic medical device
	Date of manufacture		Temperature limitation
STERILE	Sterilized		Consult instructions for use
REF	Catalogue number		Do not use if package is damaged
	Manufacturer		

Manufacturer:
Access Bio, Inc.
65 Clyde Road, Somerset
New Jersey 08873, USA.
1-732-873-4040 1-732-873-4043
info@accessbio.net
www.accessbio.net

REF G0141 **2012-12-19** IVD