TITLE PAGE

PLASMA LIPID, SERUM PROTEIN AND BODY MASS INDICES AMONG *Plasmodium falciparum* MALARIA-INFECTED WOMEN ATTENDING ANTENATAL CLINICS IN IGBO-EZE NORTH LOCAL GOVERNMENT AREA, ENUGU STATE, NIGERIA.

BY

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A PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE (M.Sc.) IN PARASITOLOGY FROM THE DEPARTMENT OF ZOOLOGY AND ENVIRONMENTAL BIOLOGY, FACULTY OF BIOLOGICAL SCIENCES, UNIVERSITY OF NIGERIA NSUKKA

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DEDICATION

To You LORD Almighty the giver of breath, energy, will, focus and commitment. Without You life and purpose is impossible. And to Engr Alozie Austine, you have been of immense and unquantifiable help to my life and dreams.

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TABLE OF CONTENTS

CHAPTER THREE: RESULTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACT	Artimisinin combination therapy
ALT	Alanine aminotransferase
ASP	Aspartate aminotransferase
BMI	Body mass index
BW	Body weight
CHD	Chronic heart diseases
Cord	Umbilical cord
CRHBP	Corticotrophin-releasing hormone-binding protein
G-6-PD	Glucose-6-phosphate dehydrogenase
GFR	Glomerular filtration rate
GPI	Glycosylphosphatidylinositol
HbF	Foetal haemoglobin
hCG	human chorionic gonadotropin
HDL-C	High-density lipoprotein cholesterol
HRP-2	Histidine rich protein-2
IE	Infected erythrocyte
IGF-1	Insulin-like growth factor-1
IGFBP	Insulin-like growth factor-binding protein
IL	Interleukin
INF-ɔ	gamma interferon
INTs	Insecticide treated bednets
IPTp-SP	Intermittent preventive treatment with sulphadoxin-pyrimethamine
IRS	Indoor residual spraying
IUGR	Intrauterine growth restriction
KAHRP	Knob-associated histidine-rich protein
LBW	Low birth weight
LC-PUFAs	Long chain polyunsaturated fatty acids
LDL-C	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
MMR	Maternal mortality ratio
NDHS	Nigeria demographic and health survey
PA	Glycerophosphate
PAI-1	Plasminogen inhibitor 1
PAM	Pregnancy-associated malaria
PC	Glycerophosphocholine
PCR	Polymerase chain reaction
PfEMP 1	*Plasmodium falciparum* erythrocyte membrane protein 1
PfEMP 2	*Plasmodium falciparum* erythrocyte membrane protein 2
PfHRP-1	*Plasmodium falciparum* histidine rich protein-1

PFHRP-2	*Plasmodium falciparum* histidine rich protein-2
PG	Glycerophosphoglycerol
PI	Glycerophosphoinositol
PIH	Pregnancy-induced hypertension
pRBCs	Parasitized red blood cells
PS	Glycerophosphoserine
RBC	Red blood cell
RDTs	Rapid diagnostic tests
RPA	Reduced plasminogen activator
SM	Sphingomyeline
TAG	Tryacyglycerol
TC	Total cholesterol
TG	Triglyceride
TNF	Tumor necrosis factor
U5	Under 5
VSA_{PAM}	Pregnancy-associated variant surface antigen
WBC	White blood cell

ABSTRACT

A cross-sectional study on the plasma lipid, serum protein and body mass indices of *Plasmodium falciparum*-malaria infected women attending antenatal clinics in Igbo-Eze North Local Government Area, Enugu State, Nigeria was conducted from August to December 2015. Weight and height of the pregnant women were measured using a beam balanced scale and a standiometer. Questionnaire was used to screen the women in compliance with outlined standard inclusion criteria. Venous blood samples for malaria diagnosis (microscopy) and plasma lipid and serum protein assays were collected from the anticubital fossa. Enzymatic and spectrophotometric methods for lipid and protein assays using Randox Diagnostic kits were employed. A total of 75 pregnant women at antenatal visit in the District Hospital consented to this study. Anthropometric measurements and malaria diagnosis were conducted on all. Those that qualified for lipid and protein assays were 72. The overall mean age was 27.60 ± 0.71 years, and body mass index (BMI), 27.65 ± 0.52 kg/m^2. Overall pregnancy-associated malaria prevalence was 50.7% (38/75) and mean parasite density 11289.74 ± 22901.73/ε L of red blood cells (RBC). The overall mean total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) were 164.12 ± 4.26mg/dl, 65.98 ± 2.54mg/dl, 159.12 ± 8.90mg/dl and 66.70 ± 4.26mg/dl respectively. The total protein (TP), albumin, globulin and albumin to globulin ratio (A/G) were 6.18 ± 0.15g/dl, 3.34 ± 0.07g/dl, 2.85 ± 0.14g/dl and 1.55 ± 0.12 respectively. Pregnancy-associated malaria prevalence in multigravidae was 57.5%, secundigradae, 50.0% and primigravidae, 38.1%. *Plasmodium falciparum* parasitaemia was significantly higher (ɟ = 0.001) in primigravidae (263.13 ± 58.23) x 10^3/ε L compared to the secundigravidae (92.14 ± 4.72) x 10^3/ε L and multigravidae (65.22 ± 20.17) x 10^3/ε L, and in the nulliparous women compared to their primiparous and multiparous counterparts (ɟ = 0.002). Parasite density varied significantly with maternal age (ɟ = 0.020). There were no significant variation in parasite prevalence and density by communities of residence. TC was significantly higher (ɟ = 0.047) in 3rd trimester (172.63 ± 5.62mg/dl) compared to the 2nd trimester (156.06 ± 5.94mg/dl) and so was TG (ɟ = 0.013) and LDL-C (ɟ = 0.036). TC was significantly lower (ɟ < 0.05) in <20 age group compared to 20 ï 29 and 30 ï 39 age group. HDL-C was significantly lower (55.76 ± 3.98mg/dl) in the 18.5 ï 24.9 BMI categories compared to the 25.0 ï 29.9 categories (74.55 ± 3.49mg/dl). TC, HDL-C and LDL-C were lower in group with pregnancy-associated malaria compared to their uninfected counterpart. Malaria infected pregnant women at the 3rd trimester had significantly lower TC (ɟ = 0.004) and LDL-C (ɟ = 0.025) concentration compared to their uninfected counterparts. Total protein, albumin, globulin and A/G showed no significant variation (ɟ > 0.05) by gravidity, parity or trimester of pregnancy. Only A/G among the protein parameters showed consistent decline in the infected though not significantly (ɟ > 0.05). Only HDL-C was significantly higher in those with >20000/ε L parasite density compared to those with < 20000/ε L. Age correlated negatively with parasite density, while TC and BMI showed a positive linear relationship with age of the pregnant women. Albumin concentration correlated positively with BMI. Usage of mosquito bednet was significantly low among the studied population (ɟ < 0.05). Pregnancy-assocated malaria induces modifications to maternal biochemical architecture that is dependent on age and duration of pregnancy. High density of *P. falciparum* in the primigravid and nulliparous women highlights the need for adequate and prompt diagnosis and treatment. It may not be necessary to suffer pregnant women with stress of fasting before their lipid assays are conducted as non-fasting lipid profile showed similar outcome to that reported for fasting by other studies.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 **Introduction**

Pregnancy, a period from conception to birth, is characterized by modification to the motherôs architecture and chemistry in order to accommodate the embryo (or foetus). This presents enormous challenges to the body of the gravid woman. The duration from conception to birth (40 weeks divisible into 3 trimesters in human) is associated with morphological, anatomical, physiological, immunological and pathological changes directed at sustaining the foetus while exacting pressure on the pregnant woman. For example, oxygen and nutrients are extracted from the maternal circulation, and metabolic byproducts, hormones and other molecules are transferred via the placenta from the foetus to the maternal circulation (Salafia and Popek, 2008). Pregnancy associated changes result from modifications to the levels of hormones such as oestrogen, progesterone and erythropoietin, and the production of hormones such as human chorionic gonadotropin (hCG). The changes induced is dependent on pregnancy stage, number of foetus and health status of the gravida (pregnant woman) (Chandra *et al.*, 2012).

The physiologic changes that accompany these hormonal changes include increased maternal fat and total water, decreased plasma protein concentration, increased maternal cardiac output, blood volume and blood flow to the kidney and uteroplacental unit, and lowered blood pressure (Costantine, 2014). Red blood cell (RBC) and white blood cell (WBC) count increases progressively from 6 ï 8 weeks of gestation and attains a plateau at approximately week 32 ï 34 (Ciliberto *et al.*, 1998). The increase in WBC count sometimes interferes with the diagnosis of infections (Pacheto *et al.*, 2013). Accompanying the approximately 20 ï 30% rise in RBC count, plasma volume increases by 40 ï 50% leading to haemodilution (Ciliberto *et al.*, 1998). The increase in blood volume facilitates exchange of substances between foetal and maternal circulation. Other physiological changes include altered activity of hepatic drug metabolizing enzymes, increased tidal volume and partially compensated respiratory alkalosis (Costantine, 2014). Maternal renal plasma flow and glomerular filtration rate (GFR) also increase progressively from the first trimester. This may lead to the loss of glucose and protein from urine (Pacheco *et al.*, 2013).

Pathologic conditions associated with pregnancy result from the combined effects of disease condition (e.g. malaria, HIV and diabetes) and immune suppressive tendency of pregnancy. Pregnancy-associated immunomodulatory activities directed at sustaining the foetus skew local placental and possibly systemic maternal immunologic responses. T helper 2-type (Th2) response which enhances humoral immunity at the expense of cell-mediated immunity is favoured during pregnancy (Jamieson *et al.*, 2006). This predisposes the gravid to infection by parasites that elicit cell-mediated immunity. The pathogen further modifies the host immunologic activities to ensure its selfish interest. These combined modifications of the body activities by pregnancy and disease conditions are further complicated by the resultant alteration of the pharmacodynamics and pharmacokinetics of drugs (Costantine, 2014).

Infection of the gravid with malaria parasites especially *Plasmodium falciparum* modifies the gravidôs systemic and placental biochemistry subjecting her to enormous physiological, immunological and pathological stress that is grossly detrimental to the well-being of the gravid and her foetus. This induced stress if not properly managed overwhelms the gravid leading to her death, abortion of the foetus (Smereck, 2011) or stillbirth (Saba *et al.*, 2008). The combined condition of malaria and pregnancy known as pregnancy-associated malaria (PAM) is a major public health concern (WHO, 2014). In sub-Saharan Africa where malaria is endemic, PAM is estimated to cause 100 000 infant mortality per annum (Guyatt and Snow, 2004), 8 ï 14% low birth weight (LBW), 8 ï 36% preterm-LBW, 13 ï 70% intrauterine growth restriction (IUGR) (Steketee *et al.*, 2001). Yet another disturbing estimate places the number of gravidae at risk of *P. falciparum* infection in sub-Saharan Africa alone at 25 million (Desai *et al.*, 2007).

The modification of placental plasma biochemical profile by placental sequestration of *P. falciparum* interferes with pregnancy-induced placental plasma biochemical configuration to the detriment of the foetus. Placental and possibly maternal systemic immunologic chemicals profile is altered by PAM stimulating foetal abortion (Saba *et al.*, 2008; Maestre and Carmona-Fonseca, 2014). Systemic plasma biochemical changes induced by PAM compromise the availability of important metabolites to foetus (Brabin *et al.*, 2004; Guyatt and Snow, 2004). Very recently, Ayoola *et al.* (2012) reported PAM-induced significant ($P < 0.05$) lowering of maternal total

cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). The lowering of TC and LDL-C was very significantly correlated with birth weight ($P<0.001$) (Ayoola *et al.*, 2012). They also reported increase in maternal tumor necrosis factor (TNF) and observed that umbilical cord blood insulin and insulin-like growth factor-1 (IGF-1) correlated with birth weight.

Pre-pregnancy body mass and weight gain during pregnancy is known to be an important measure of maternal nutrition, and a major determinant of foetal and neonatal survival, neonatal body weight (BW) and childhood and adulthood height, health and cognitive ability (Adair, 2007; Ludwig *et al.*, 2013; Coffey, 2015). Weight gain or reduction induces changes in plasma lipid profile. How malaria interacts with plasma biochemical and body mass index (BMI) in PAM may present interesting prospects in the interpretation of pregnancy outcomes.

1.1.1 Justification of Study

Approximately 25 million pregnant women are at risk of *P. falciparum* infection in sub-Suharan Africa alone (Desai *et al.*, 2007), where it is also estimated that 100 000 infants die annually from pregnancy-associated malaria (Guyatt and Snow, 2004). In areas of endemicity, malaria is associated with low birth weight (LBW), preterm-LBW and intrauterine-LBW. Spontaneous abortion during pregnancy-associated malaria is believed to be associated with placental and possibly systemic plasma protein changes. Plasma lipid changes associated with pregnancy-associated malaria may interfere with nutrient transfer to foetus from maternal circulation which may compromise child growth leading to LBW. Changes to plasma lipid and proteins may alter drug pharmacodynamics and pharmacokinetics. More so, there is growing evidence for the reliability of non-fasting lipid profile in lipid indexing. Thus, the assessment of pregnancy-associated malaria induced plasma biochemical changes is a multi-barreled arsenal in combating pregnancy-associated malaria. There is, however, paucity of information on malaria-induced modification of plasma lipid and serum protein by *P. falciparum* during pregnancy and how such can be used with BMI as predictor of pregnancy outcome. This informed this research which sought to investigate the changes to serum protein, plasma lipid and BMI among *falciparum*-

malaria infected pregnant women in Igbo-Eze North Local Government Area, Enugu State, Nigeria.

1.1.2 Study Objectives

The general objective of the study was to assess the plasma lipid, serum protein and body mass indices among *falciparum* malaria-infected women attending antenatal clinics in Igbo-Eze North Local Government Area, Enugu State, Nigeria. Specifically, this study sought to determine the

1. Body mass index (BMI) of the pregnant women with malaria and those without;
2. Prevalence and intensity of *falciparum* malaria among women attending antenatal clinics in this region;
3. indices of serum protein and plasma lipid of interest among pregnant women with and without malaria; and
4. Comparison of the measured variables of interest by pregnancy characteristics, and among women with and without malaria.

1.2 Literature Review

1.2.1 Malaria: burden, transmission, symptom, diagnosis and control

Malaria, an acute febrile illness caused by protozoa of the genus *Plasmodium*, has afflicted mankind since antiquity. Presently, malaria poses enormous burden to societies, especially to the developing countries. It is especially regrettable in sub-Saharan Africa. In Nigeria, over 12.0 percent of the gross domestic product (GDP) is expended on malaria annually (Jimoh *et al.*, 2007). The estimated annual costs (in millions) for the management of malaria in Ghana, Kenya and Tanzania in 2013 were placed at US$37.8, US$ 109.0 and US$ 131.9 respectively (Sicuri *et al.*, 2013). This enormous cost of managing malaria further contributes to the negating of development efforts in these regions. Globally, an estimate of 198 million cases of malaria with approximate death of 584 000 people were recorded in 2013 (World Health organization [WHO], 2015). The most vulnerable groups from these estimates are children under five (U5) and pregnant women.

Ninety-seven countries and territories recorded ongoing cases of malaria transmission as at 2014 (WHO, 2015). Thirty countries in sub-Saharan Africa account for 90% of global malaria deaths. The disease is endemic in Africa, Central and South America, South, Southeast and Middle Eastern Asia, Eastern Europe and Southern Pacific (Figure 1). Nigeria, Democratic Republic of Congo (DRC), Ethiopia and Uganda accounts for nearly 50% of mortalities (Nigeria Malaria Fact Sheet [NMFS], 2011; Presidentôs Malaria Initiative Africa Indoor Residual Spraying Project [PMI AIRS], 2015). About 40% of death ascribable to malaria occurs in Nigeria and Democratic Republic of Congo (DRC) (WHO, 2013). The entire population of Nigeria (approximately 182 million) is at risk of malaria which accounts for 60% of outpatient visits and 30% of hospitalizations, and 11% of maternal mortality annually (PMI, 2015).

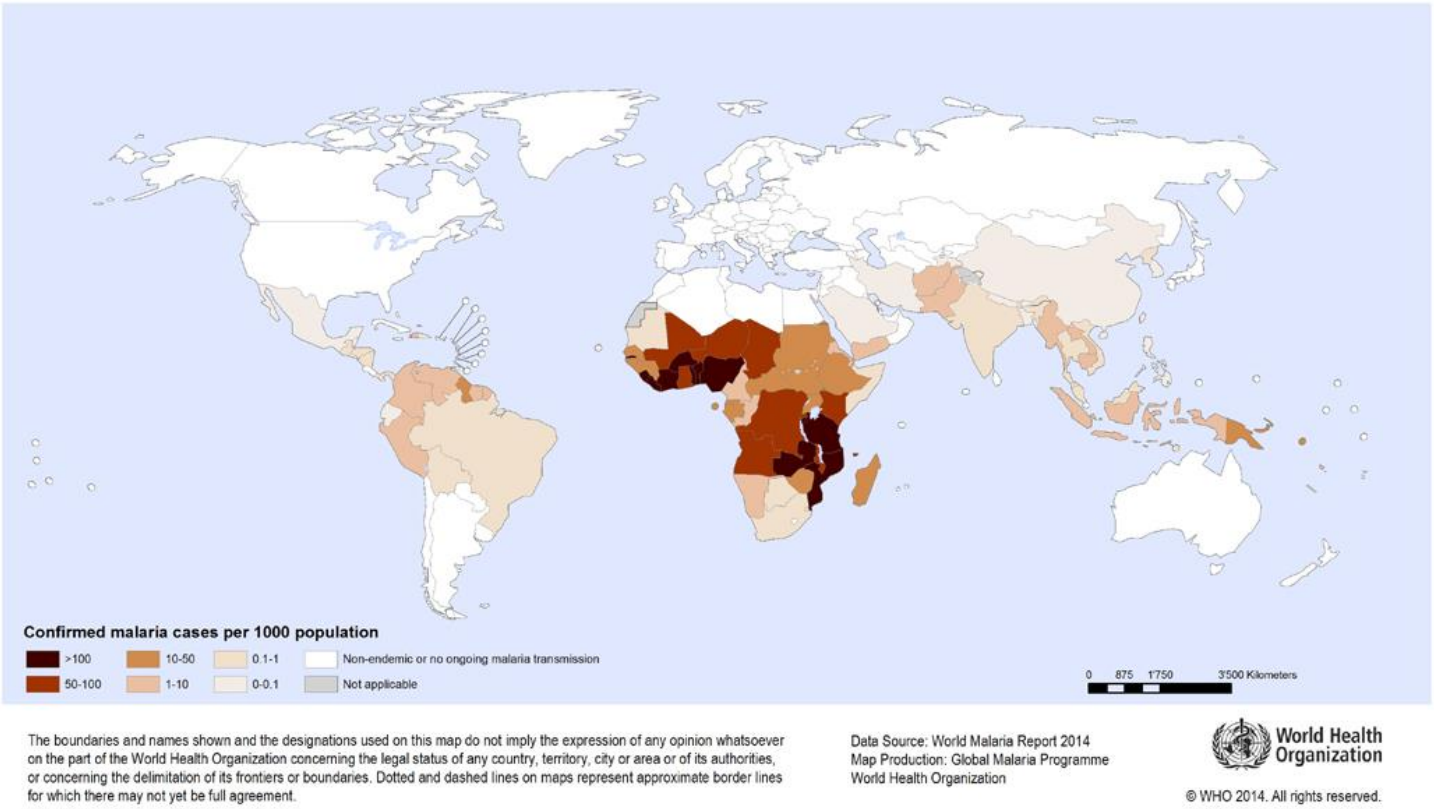

Figure 1: Classification of countries by stage of elimination of malaria, 2013
Source: WHO (2016)

Five species of the genus *Plasmodium* are pathogenic to human, namely, *P. falciparum, P. vivax, P. malariae, P. ovale* and the recently included *P. knowlesi*. *P. falciparum* causes the most virulent cases of human malaria. It is of a major burden to Nigeria (Figure 2). The anthropophilic, haemophagic and nocturnal female mosquitoes of the genus *Anopheles* transmit the haemoprotozoa *Plasmodium* by inoculation into peripheral blood of the human host during blood meal. Forty species of *Anopheles* transmit anthropogenic and zoonotic malaria (Malaria Atlas Project [MAP], 2015), but *Anopheles gambiae, Anopheles funestus, Anopheles nili* and *Anopheles moucheti* are the primary vectors of human malaria in Africa and the most efficient in the world (Sinka *et al.*, 2010; Onyido *et al.*, 2014) (Figure 3). Transmission of human malaria is high where infected mosquitoes live in close proximity to man, have long lifespan and high preference for human blood over that of other animals (WHO, 2015). Rainfall, humidity and temperature also affect survival of mosquitoes and the transmission of *Plasmodium.* In Nigeria, the peak of transmission is usually during and just after the rainy season.

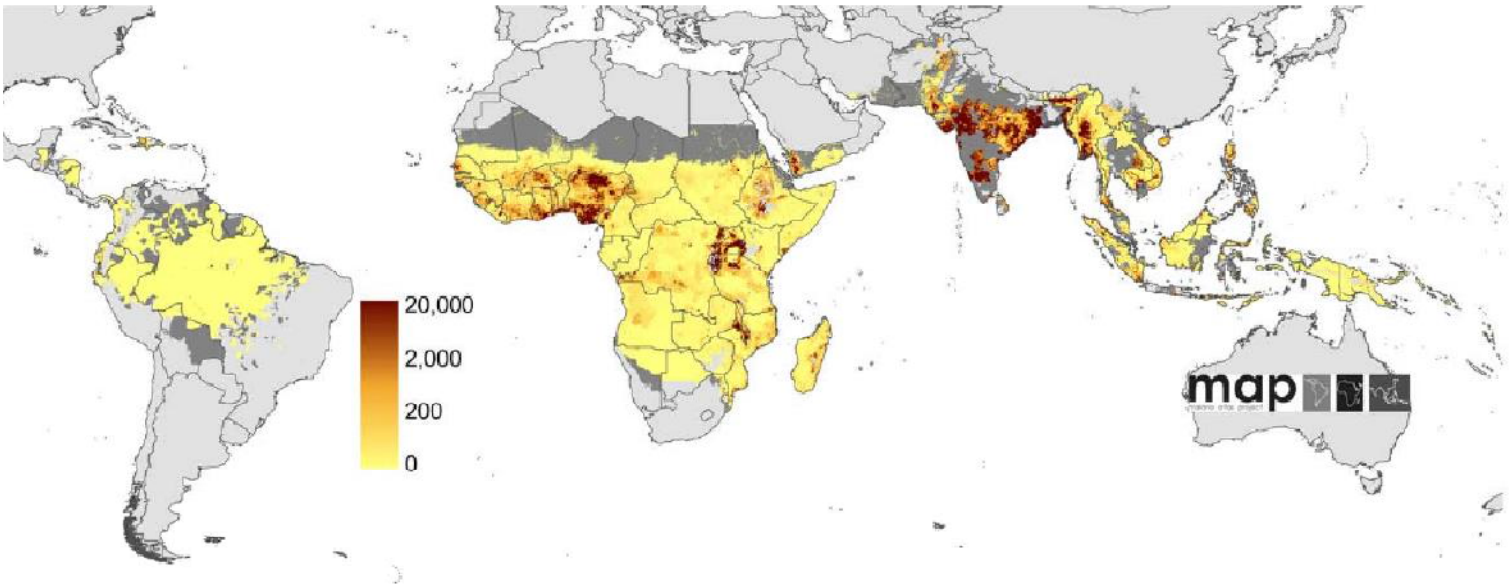

Figure 2: Map showing the global clinical burden of *Plasmodium falciparum*
Source: Hay *et al.* (2010)

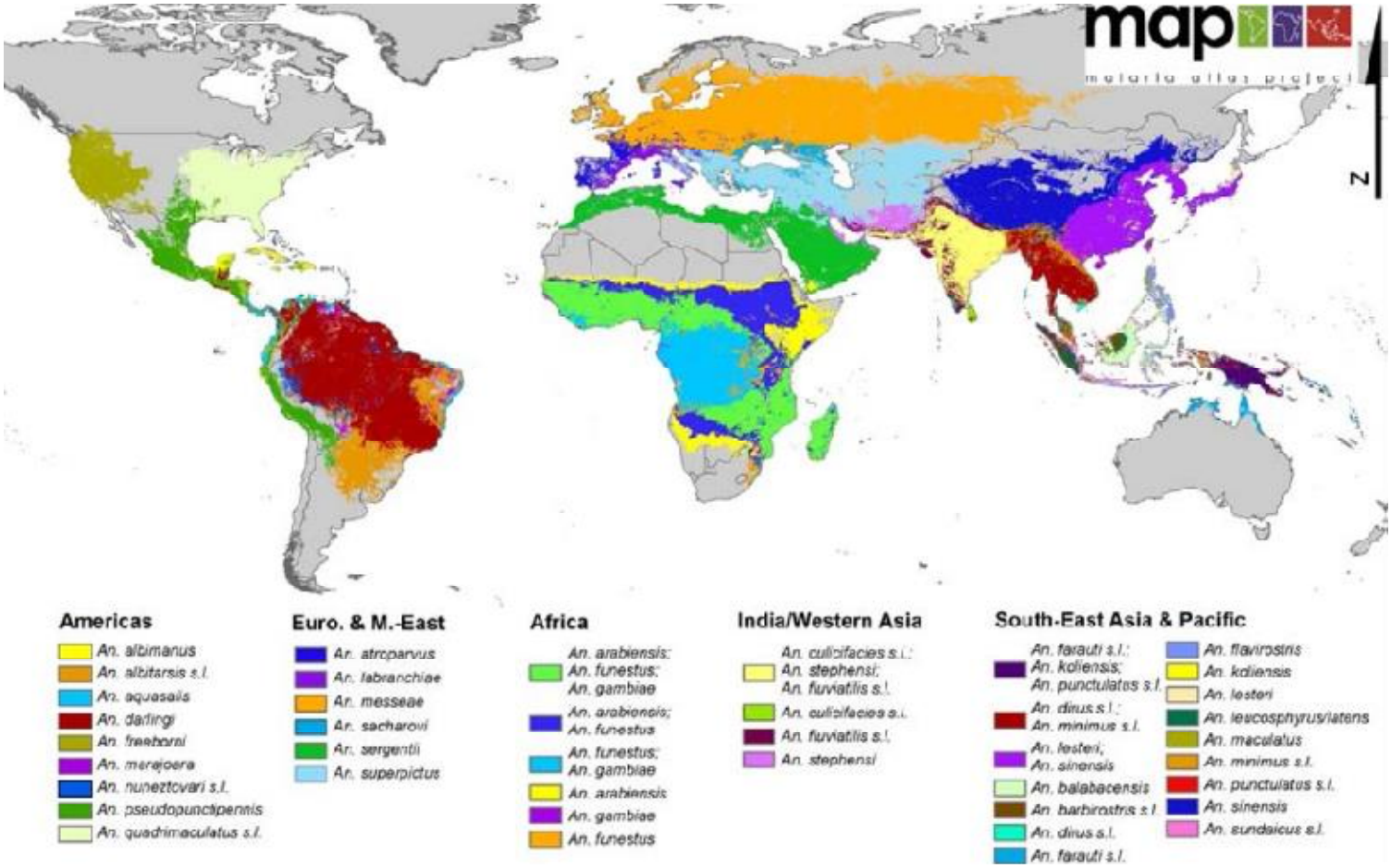

Figure 3: A global map of dominant malaria vectors
Source: Sinka *et al.* (2012)

The life cycle of *Plasmodium* parasite in human malaria is two phases: hepatocytic (exoerythrocytic) and erythrocytic (Figure 4). Immediately after inoculation into human during mosquito blood meal, the sporozoites are carried by the circulation to the liver within 15 ï 45 minutes of inoculation, where they invade the hepatocytes. In the hepatocytes, they proliferate asexually into thousands of merozoites which are released by the rupturing of the hepatocytes. The development of sporozoites into multicellular, large (30 ï 70µm) meront (or schizont) takes about 6 days for *P. falciparum* to 15 days for *P. malariae* (Eckert, 2005). Merozoites released from the liver tissue schizonts infect the erythrocytes initiating the erythrocytic phase of the life cycle. *P. ovale* and *P. vivax* may retain hypnozoites as dormant forms in the hepatocytes which may be reactivated to initiate erythrocytic cycle months or years after initial infection.

By the aid of appropriate receptors, merozoites invade red blood cells (RBCs) where these small (1.5 µm), ovoid forms develop first into rings and then into late forms (trophozoites and schizonts). Schizont-ladden RBCs rupture to release more merozoites that infect the erythrocytes repeatedly (Eckert, 2005; Tripathy and Roy, 2014). Micro- and macrogametocytes released during the erythrocytic cycle may be picked up by mosquitoes during blood meal initiating the arthropod phase of the cycle. In the female anopheline mosquito, the microgametocytes (by

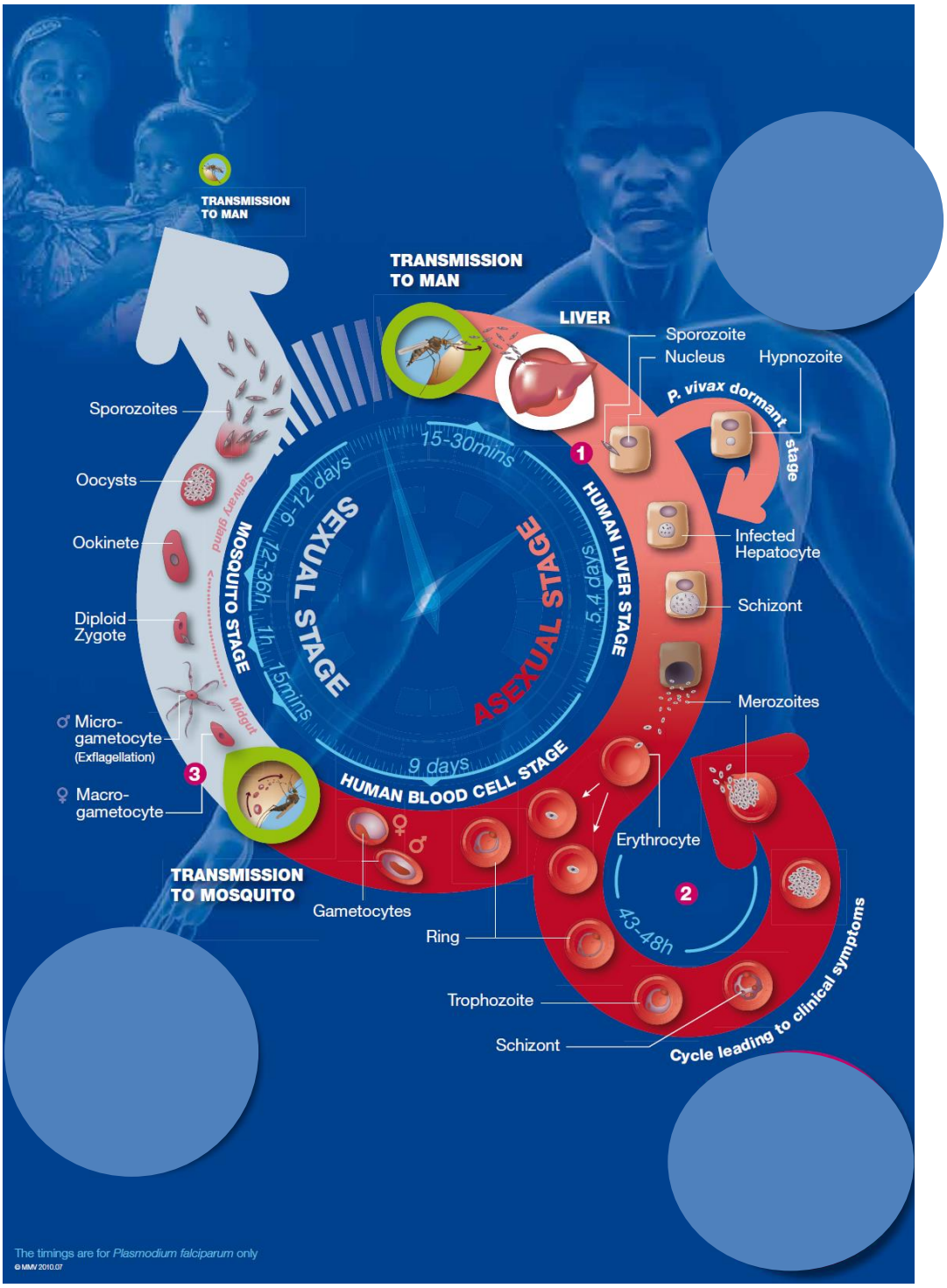

Figure 4: Life cycle of *Plasmodium* parasites
Source: Medicine for Malaria Venture (2010)

exflagellation) and macrogametocytes mature into microgametes and macrogametes respectively. A microgamete swims about, locate, penetrate and fertilize a macrogamete which develops into a motile ookinete which measure about 10µm ï 12µm in length (Cox, 2004; Roberts and Janovy Jr, 2010). Ookinetes penetrate the peritrophic membrane of the insect gut into the haemocoel where they transform into oocytes that eventually give rise to sporozoites. The sporozoites break out of the oocytes, migrate round the body and enter the salivary gland from where they may be transmitted to human host during blood meal.

In the erythrocytic stage, the mechanical properties of the *Plasmodium* parasitized RBCs (pRBCs) are altered significantly. The modification to RBC architecture favours parasite sustenance to the detriment of the host. Parasite nutrients and metabolites such as amino acids, lipids, carbohydrates and purines are recruited through special channels created in the pRBCs membrane (Chen *et al.*, 2000; Rub *et al.*, 2013). Parasite-derived polypeptides including *P. falciparum* erythrocyte membrane protein 1(PfEMP 1), rosettin/rifins and knob-associated histidine-rich protein (KAHRP) are inserted into and protrude from RBC membranes (Chan *et al*., 2000; Wickham *et al.*, 2001). These molecules participate in knob formation (Leech *et al.*, 1984) which are important in rosette formation and sequestration of pRBCs, a major determinant of severe malaria (Chen *et al.*, 2000; Wickham *et al.*, 2001; Zhang *et al.*, 2015). The binding of pRBCs to uninfected RBCs (rosetting) and the adherence of pRBCs to vascular endothelium (sequestration) by knobs and knobless RBCs may lead to occlusion of blood vessels and subsequent destruction of blood cells and impaired oxygen delivery. Massive sequestration in the brain is believed to cause cerebral malaria (Chen *et al.*, 2000).

Symptoms associated with malaria depend on factors related to the parasite and the host. In non-immune individuals, symptoms appear less than two weeks after the effective mosquito bite. The first symptoms are fever, chills, vomiting and headache which may progress abruptly, in case of *P. falciparum* infection, into life threatening morbidity within 24 hours if untreated (WHO, 2015). Multi-organ involvement, severe anaemia, respiratory distress syndrome and cerebral malaria are associated with severe malaria. The contributory activities of host-derived and parasite-derived factors define malaria outcome. Malaria parasite antigens or toxins stimulate at a varying scale the production of host cytokines which may stimulate inflammatory responses enhancing cytoadherence and the inherent deleterious outcome. Glycosylphosphatidylinositol

(GPI)-anchored proteins (parasite-derived toxins) released by malaria parasites late stages (trophozoite and schizont stage) may directly damage host tissues or stimulate overproduction of cytokines (Chen *et al.*, 2000; Proellocks *et al.*, 2007). Overproduction of cytokines such as tumor necrosis factor-Ŭ (TNF-Ŭ), gamma interferon (INF-ɔ), and interleukin 1(IL-1), unlike their moderate production which is important for human immunologic response to combat parasites, elevate morbidity (Chen *et al.,* 2000). TNF-Ŭ causes fever (Stefferl *et al.*, 1996). TNF-Ŭ and INF-ɔ upregulate endothelial receptor and probably enhance cytoadherence of pRBCs (Mackay *et al.*, 1993; Chen *et al.*, 2000).

The prompt detection of the parasites in the circulation and prompt treatment is very important to the survival of the subject. In malaria-endemic areas, treatment according to recommendation should commence within 24 hours after appearance of symptoms. Suspected cases should be confirmed using parasite-based diagnostic testing (CDC, 2015a). The detection of *Plasmodium* trophozoites in peripheral blood by the aid of microscope (malaria microscopy) is the ógold standardô test for malaria. This involves fixing of thin and/or thick blood smear collected from subject, staining it with appropriate dye (mostly Giemsa) and examining slide for *Plasmodium* using a microscope. The faster and less technical rapid diagnostic tests (RDTs) which involve the detection of *Plasmodium*-derived antigens or anti-plasmodial antibodies in the blood of the subject are used in many clinics for malaria tests. The malarial antigens targeted by most RDTs are the histidine-rich protein 2 (HRP-2), *Plasmodium* aldolase, and parasite lactate dehydrogenase (Gatti *et al*., 2007). Another diagnostic technique, the polymerase chain reaction (PCR)-based assay is built on the principle of detection of parasite deoxyribonucleic acid (DNA) in subject. For malaria parasite, most PCR-based assays detect the presence of 18S ribosomal ribonucleic acid (RNA) (18S rRNA) genes (Gatti *et al*., 2007). Plasma lipid (Al-Omar *et al.*, 2010; Akanbi, 2013; Chikezie and Okpara, 2013; Neves *et al.*, 2013) and possibly serum protein (Fisayo, 2007; Abdagalil and ElBagir, 2009) changes are prospective diagnostic tools for malaria.

The control of malaria has come with it challenges. A multidimensional approach to malaria control encompassing timely prophylaxis, good management of clinical cases and effective vector control strategies, vaccine research, and training and education on malaria prevention

(Carrington, 2001) are the present control strategies employed against malaria. Effective management of clinical malaria is defined variously and constantly reviewed in consideration of dynamics of disease outcome, and variation of burden in geographical areas. Monotherapy with chloroquine, quinine and arthemeter are beset with problems of resistance by malaria parasites. This has led to the current recommendation of combination therapy in malaria control. A combination of artemisinin-based compounds with other anti-plasmodial drugs such as mefluoquine, amodiaquine, lumefantrine piperaquine and sulfadoxine/pyrimethamine, commonly called arteminsinin combination therapy (ACT) is effective against malaria. The WHO (2012a) released a series of documents promoting the 3Ts (Test, Treat and Track) in effective malaria management in malaria-endemic countries. This initiative which is directed at achieving universal diagnostic coverage and strengthening malaria surveillance system is based on diagnostic testing, antimalarial treatment, and tracking back home of the individual receiving the treatment for the purpose of testing the household for malaria. The financial cost of this approach in sub-Saharan Africa where poverty is widespread (Baingana and Bos, 2006, SESRTCIC, 2007; Hunger Notes, 2016) may not make its implementation feasible. The high cost of artemesinin-based combination therapy and pre-treatment diagnosis by RDTs or microscopy subject the poor from malaria endemic regions to further financial burden. Even though the implementation of RDTs before malaria has been reported to be more cost-effective than presumptive treatment (Shillcut *et al.*, 2008; Uzochukwu *et al.*, 2009), the additional cost incurred by tracking home and subsequent treatment of household if need be may not support such laudable approach.

Vector control strategies include active destruction of the mosquito vectors by the use of insecticides and biological larviphagal organisms and screening of human against malaria. Widespread adoption of insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) have been effective in reducing malaria in endemic countries (Nyarango *et al.*, 2006; Igwe *et al.*, 2007; Osundu and Jerome, 2009). The development of resistance to insecticide-based approaches by vectors is driving research into development of other approaches to malaria control such as the release of sterile male mosquitoes (Baeshen *et al.*, 2014).

1.2.2 Serum protein and plasma lipid indices

The plasma is the fluid medium that convey the formed elements-erythrocytes, leukocytes and platelets, around the body. It is an aqueous solution consisting of 90% water, 7 ï 8% soluble proteins, 1% inorganic salts, and the rest of organic compounds of disparate origin such as lipoproteins, amino acids, vitamins, hormones, and other elements on transit (Jacqueira *et al.*, 1986). The composition of the human plasma is an essential diagnostic parameter able to provide comprehensive result on the health of an individual (Deutsch *et al*., 2005). The ease with which the plasma can be safely obtained and its ability to provide a comprehensive snapshot of the human physiological state has made it an attraction for disease diagnosis (Anderson and Anderson, 2002).

Serum protein index

The serum protein index is a subset of the plasma protein index. Removal of prothrombin, fibrinogen and other clotting factors from the plasma reduces it to serum. Thus, the plasma gives a better picture in terms of protein contents than the serum. The human plasma protein has served enormous diagnostic purposes and still holds elating promises in disease diagnosis and therapeutics (Anderson and Anderson, 2002). The plasma in addition to the classical plasma protein, those that carry out their function within the circulation (Putman 1975 ï 1978 in Anderson and Anderson, 2002) contains proteins that leak into or are secreted by tissues or foreign organisms (Anderson and Anderson, 2002; Deutsch *et al.*, 2005). This lead Anderson and Anderson (2002) to classify the plasma protein on the bases of function, source, structure and duration in circulation into the following categories:

- Proteins secreted by solid tissues that act as plasma: the classical plasma proteins are largely secreted by the liver and intestine. They have molecular mass larger than the kidney filtration cutoff (~45kDa) and hence longer residual time in circulation.
- Immunoglobulins (Igs): Igs function in the plasma but because of their complexity of structure and diversity are considered separately.
- ñLocalò receptor ligands: the cytokines and other short distance mediators of cellular response fall into this category. Their molecular mass is less than the kidney filtration cutoff, thus they have short residence time in circulation.

- ñLongò distance receptor ligands: here are the classical hormones and peptides.
- Temporary passengers: these include transient transversers of the plasma such as lysosomal proteins.
- Tissue leakage products: these proteins normally function within cells but death or damage of cells may lead to their discharge into the plasma. They are important diagnostic markers.
- Aberrant secretions: these proteins released from damaged tissues are also important diagnostic markers. Their release is presumed to be a normal functional activity of their source tissue.
- Foreign proteins: these are released by endoparasitic organisms.

The exact number of proteins present in the plasma is presently unknown. Conservative estimates have reported numbers such as 300 (Anderson and Anderson, 2002) and 150 (Escher *et al.*, 2014). Some of the proteins reported include: albumin, globulin, alanine aminotransferase (ALT), adiponectin, alkaline phosphatase, acid phosphates, aspartate aminotransferase (AST), immunoglobulins (Igs), cytokines, luteinizing hormones (LH), insulin-like growth factor-binding protein (IGFBP), corticotrophin-releasing hormone-binding protein (CRHBP), glucose-6-phosphate dehydrogenase (G-6-PD), human chorionic gonadotropin Beta (hCG) and von Willebrand Factor. They vary in abundance with only 50 accounting for 90% of all plasma protein (Tu *et al.*, 2010).

Even though some few plasma proteins have been widely used for diagnostic purpose, all plasma proteins probably are significant as diagnostic. Disparity in plasma protein concentrations arising from difference in genetic make-up (Monteiro *et al.*, 2014), disease (Abdagalil and Elbagir, 2009; Hye *et al.*, 2014), exercise (Dahlqvist *et al.*, 2014), smoking (Yigiter *et al.*, 2006), age (Ignjatoric *et al.*, 2011; Monteiro *et al.*, 2014), time of day (Svedman *et al.*, 2002), gender (Ignjatoric *et al.*, 2011; Monteiro *et al.*, 2014), diet (Reseland *et al.*, 2001), season (Smith *et al.*, 1999), sleep (Heinzlmann *et al.*, 2014) and obesity (Tchernof *et al.*, 2002) are important factors to consider in plasma proteome based diagnosis.

Plasma lipid index

Lipids are of structural and physiological significance in living organisms. In biological systems, these water-insoluble substances act as structural elements of biological membranes, enzyme cofactors, electron carriers, hydrophobic anchors for proteins, light-absorbing pigments, emulsifying agents in the digestive tract, hormones and molecular messenger (Nelson and Cox, 2005). They exist as different molecular species grouped into categories based on structural differences and constituent chemical groups. In the human plasma, these molecular species are categorized as fatty acyls, glycerophospholipids, sphingolipids, sterols, glycerolipids and prenols. Even though there is disparity in concentration and number of these different categories of lipids molecules in the human plasma, inter-relationship in biosynthetic processes exist for all (Quehenberger *et al.*, 2010) (Figure 5).

Quehenberger *et al.* (2010) reported as much as 500 distinct molecular species in the plasma of healthy human distributed into the categories shown in Figure 5.There is, however, a constant flux in the component lipids and their concentration in the plasma (Christie, 2014) but homeostatic regulation exist. This constant flux may be related to functional role and biosynthetic pathways of the lipids. Pathologic and physiological state and genetic make-up of an individual contribute (Deeg, 2006; Elmehdawi, 2008; Ayoola *et al.*, 2012). Age, gender, posture and chemotherapy are other contributors to plasma lipid changes.

Plasma fatty acids are a highly metabolically active lipid class mainly derived from adipose tissue. Oleic acid (18:1), palmitic acid (16:0) and stearic acid (18:0) are the most abundant fatty acids making up about 78% of free fatty acids in the plasma (Quehenberger *et al.*, 2010). Tryacyglycerol (TAG) is the most abundant member of the glycerolipid category in normal human plasma (Quehenberger *et al.*, 2010). The concentration is greatly dependent on food intake such that the postprandial concentration of TAG (or TG) is much higher than the fasting concentration (Nigam, 2011). The increase is as a result of the synthesis and packaging of TAG into lipoprotein basically chylomicrons and very low density lipoprotein (VLDL) is a key process in the distribution of lipids to body tissues (Quehenberger *et al.*, 2010). In a comprehensive analysis of the human plasma lipidome, Quehenberger *et al.* (2010) also reported the presence of glycerophosphate(PA), glycerophosphocholine (PC), glycerophosphoinositol (PI),

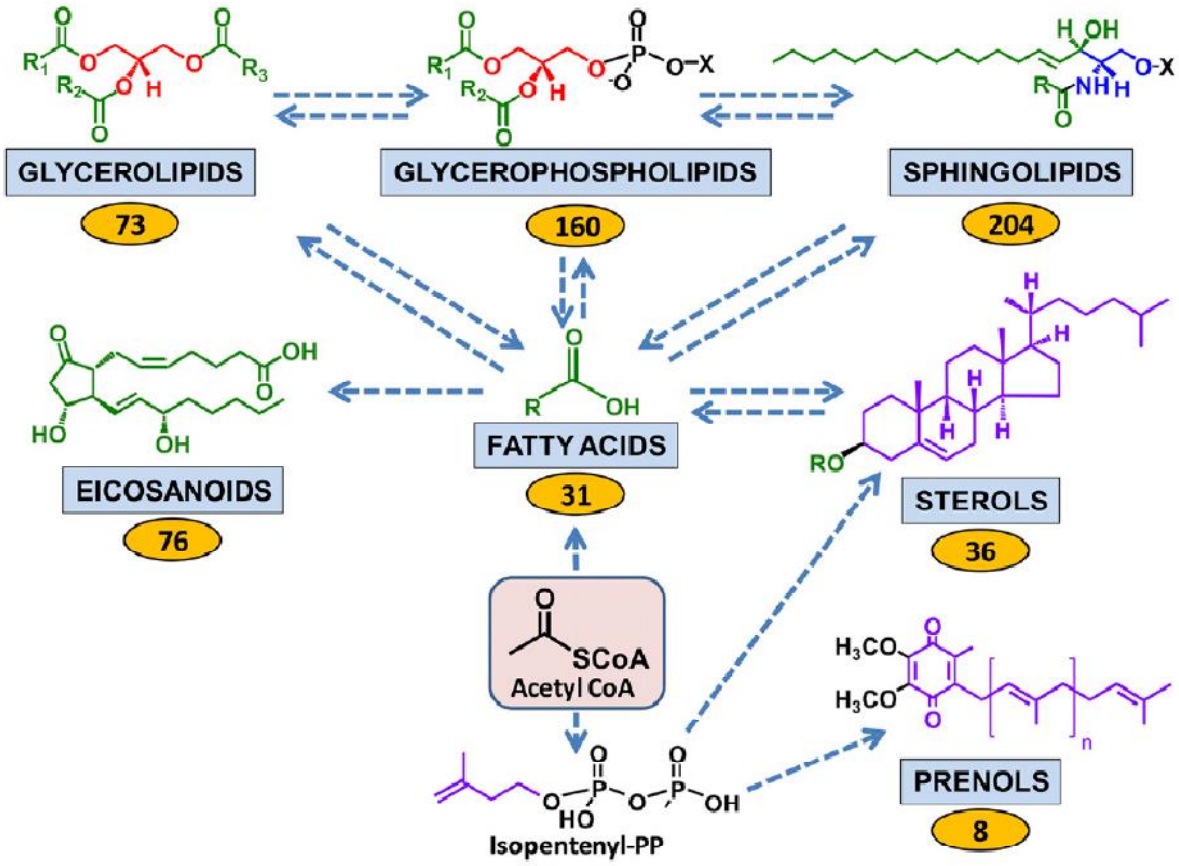

Figure 5: Human plasma lipid diversity and the numbers of the different molecular species in each category. The numbers in the oval below each category represent the numbers reported. Source: Quehenberger *et al.* (2010).

glycerophosphoglycerol (PG) and glycerophosphoserine (PS) species (glycerophospholipids); cholesterol, demosterol, lathosterol, 7-dehydrocholesterol, campesterol and cholesterol esters (sterols); sphingomyeline (SM), sphingodiene, sphinganine and lactosylceramide (sphingolipids); and dolichols and ubiquinones (prenols).

Structural modifications in the lipid categories and species relate to functional roles in normal and abnormal physiological state. The lipoproteins such as the VLDL-cholesterol, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) are complexes of proteins and lipids that render the lipids structurally compatible with the aqueous environment of the body fluid in which they are transported. Also, the smaller molecular size of the free fatty acids facilitates their trasplacental supply to foetal circulation (Herrera *et al.*, 2006).

Assessment of the lipid contents of the plasma (plasma lipid indexing) is of important diagnostic and therapeutic significance. Triglyceride, HDL-C and LDL-C are widely used in the diagnosis of diseases (e.g chronic heart diseases, CHD). But there is a present surge in the profiling of both human lipidome (Lipidemics) and proteodome (Proteonomics) to accompanying the Genomics in the diagnosis of metabolic diseases. More so, the diagnostic significance of other neglected plasma lipids and proteins is being explored. The modifications in plasma lipids and proteins profile are presently of interest in the research into pregnancy-associated malaria (PAM).

1.2.3 Body mass index (BMI)

BMI is a simple, safe, cost-effective and non-invasive estimate for body fat. It is a measure based on observed correlation between adiposity and the ratio of the weight of an individual and its height (key *et al.*, 2014). It is calculated as weight in kilograms divided by the square of height in meters (kg/m^2). As implied by the mode of calculation, BMI is an indirect measure for body fat. It finds application as an indirect method of assessment of an individualôs predisposition to fat-associated health risks (Charbonneau-Roberts, 2005; CDC, 2011, 2015b). For example, Type 2 diabetes mellitus and cardiovascular diseases are associated with overweight and osteoporosis with underweight.

BMI relationship to body fat is affected by factors such as sex, age and muscle mass. Women tend to have greater amount of total body fat than men of equivalent BMI; older adult, on average tend to have more body fat than younger adults with same BMI; while muscular individuals may have increased BMI resulting from the additional muscles (CDC, 2011). Other limitations to the use of BMI in the assessment of body fat is it inability to determine regional body fat distribution (Kok *et al.*, 2004) and unsuitability in conditions where individuals have long or short legs to torso length (Abou-Hussein *et al.*, 2011). Even with these challenges, BMI is still reliable as it correlates to more direct measures of body fat such as dual energy x-ray absorptiometry and underwater weighing (CDC, 2011). Modifications such as sitting height-to-standing height ratio (SH/S) where the individualôs weight to sitting height (i.e. length as normally measured, but this time while the individual is sitting upright) ratio is utilized as correction for long/short leg to torso length associated shortcomings.

Weight status of individuals using BMI is determined by comparison of an individualôs BMI to that of globally accepted range. This is such that BMI below 18.5 is underweight, 18.5 ï 24.9 normal, 25.0 ï 29.9 overweight and 30.0 and above as obese.

1.2.4 Pregnancy-associated malaria (PAM)

Infection with malaria during pregnancy is a major public health concern constituting a serious risk to the pregnant woman, her foetus and the newborn (WHO, 2014). This is as infection with malaria parasites especially *P. falciparum* further subject the gravida (pregnant woman) to physiologic, immunologic and pathologic stress in addition to that arising from pregnancy. This combination of stresses which on one hand is directed at ensuring foetus survival and on the other at sustaining parasite, may overwhelm the pregnant woman leading to her death, or abortion of the foetus (Smereck, 2011), still birth (Saba *et al.*, 2008) and low birth weight (LBW) neonates (Schantz-Dunn and Nour, 2009). Saba *et al.* (2008) reported as high as 14%, 9%, 6% and 30% cases of spontaneous abortion, neonatal death, preterm labour and puerperal pyrexia respectively that resulted from malaria infection during pregnancy among Pakistanis. Wrong

drug administration to the gravid woman may have teratogenic consequences if not death to the foetus. Thus, malaria in pregnancy is of critical concern.

The pathology in pregnancy-associated malaria (PAM) is dependent on the localization of the parasite in the maternal circulation or maternal-foetal interface and the immunologic response elicited. This probably led to the consideration of PAM under the contexts: gestational, placental and congenital (Maestre and Carmona-Fonseca, 2014). Where gestational malaria was interpreted as the presence of *Plasmodium* sp. in maternal circulation which eventually lead to the distribution of parasitaemia to the placenta. Thus, this may be considered to encompass placental localization as was used by Fisayo (2007). Congenital malaria implies neonate peripheral blood isolation of parasites. In this review focus was given to gestational malaria as encompassing maternal circulating, placental and umbilical cord (hereafter used as ócordô) localization of *Plasmodium* sp. with little consideration of outcomes post delivery.

Immunity against *Plasmodium* requires both cell-mediated and humoral immunity. The pre-erythrocytic stage is generally accepted to be controlled by cell-mediated mechanism while humoral activity ensures immunity against the erythrocytic. But the body is never able to develop effective immunity against the parasite due to the ability of the parasite to express variant surface molecules (Petter *et al.*, 2007). In malaria endemic areas some levels of acquired immunity protect both pregnant and non-pregnant adults.

The control of parasitaemia during pregnancy presents a major challenge to the gravid woman. Unlike in the non-pregnant where the different aspects of immunologic responses ï humoral and cell-mediated arms, may be employed in the control of parasitaemia, the tendency of response to skew to the humoral arm during pregnancy may have deleterious consequences. While the maintenance of humoral response characterized by the production of T-helper type 2 (Th2) cytokines such as interleukin(IL)-4, IL-5 and IL-13 is important for the sustenance of the foetus, it may not effectively clear parasitaemia. This condition is similar to that utilized by helminthes for continual survival facilitating chronic infection in host (Maizels and Yazdanbakhsh, 2003; Moreau and Chauvin, 2010), where even though such humoral-tilted modulation is effective in prevention of pro-inflammatory response and destruction of pathogens controlled by humoral

responses, those controlled by cell-mediated immunity thrive. It has, however, been observed that *P. falciparum*-associated pregnancy is characterized by the sequestration of the parasite to placenta which depending on burden may elicit cell-mediated immunological response. This response characterized by placental localized production of pro-inflammatory or Th1 cytokines such as interferon (INF)-ɔ, tumour necrosis factor (TNF)-Ŭ, IL-12 and IL-2 may lead to placental tissue damage, spontaneous abortion of foetus, preterm labour and intrauterine growth retardation (Ayoola *et al.*, 2012; Maestre and Carmona-Fonseca, 2014).

This shows that critical regulation of systematic and local immunologic response is important during pregnancy. It is no doubt why it has been observed that maternal cytokines and soluble cytokine receptors level changes throughout the course of pregnancy. Studies have observed that during pregnancy, maternal systemic circulating levels of IL-4, IL-6, IL-10 and IL-13 (Th2 cytokines) increase progressively while the plasma concentration of IL-1Ŭ, IL-1ɓ, IL-2, IL-12 and INF-ɔ (Th1 cytokines) take an opposite course even becoming significantly less in the third trimester than that observed in the first (Shimaoka *et al.*, 2000; Sykes *et al.*, 2012). TNF-Ŭ level in the maternal circulation remain unchanged though soluble tumor necrosis factor receptor (sTNFR) level increases which may interfere with the activity of TNF-Ŭ probably preventing any negative consequences for the foetus (Thévenon *et al.*, 2010; Mastrae and Carmona-Fonseca, 2014). Placenta localized immunologic response is also Th2 arm directed (Jakobsen *et al.*, 1998). These modifications are attributable to the upregulation of hormones such as progesterone, oestrogen, testosterone and cortisol during pregnancy (Robinson and Klein, 2012).

In areas where malaria is endemic, it has been observed that while children above 6 months suffer severe malaria with fatal consequences if left untreated, adults irrespective of sex tend to acquire partial immunity with persistent exposure to *Plasmodium* (Sauerzopf *et al.*, 2014). This immunity acquired is characterized by asymptomatic parasitaemia. In primigravidae and secondegravidae, this acquired partial immunity appear to decline or is completely ineffective (Jamieson *et al.*, 2006; Maestre and Carmona-Fonseca, 2014) such that *P. falciparum* infection if not carefully managed develop into severe disease state threatening the life of the foetus and its carrier.

The ability of *P. falciparum* to express on its surface pregnancy-associated variant surface antigen (VSA_{PAM}) which mediate binding of *P. falciparum* infected erythrocyte to chondroitin sulphate proteoglycans in the placental intervillous space define morbidity (Fried and Duffy, 1996; Viebig *et al.*, 2005; Nielsen *et al.*, 2007). VSA_{PAM} also known as VAR2CSA (product of the *var2csa* gene family) is a conserved member of the *P. falciparum* erythrocyte membrane protein 1(PfEMP1). Its association with severity of malaria in primigravidae and secondigravidae has been corroborated by observation of poor anti-VSA_{PAM} antibodies in men and children (Salanti *et al.*, 2003). Anti-VSA_{PAM} specific IgG antibodies increase with number of pregnancies such that sequestration of *P. falciparum*-infected erythrocytes to placental chondroitin sulfate A is eventually blocked (Khattab *et al.*, 2004; Taylor *et al.*, 2004). It is the sequestration of *P. falciparum* to placental chondriotin sulfate A that characterize severe morbidity and possible mortality and other inherent deleterious consequences of PAM. Additionally, *var2csa* gene occur more in infected placentae than in samples of *P. falciparum* from non-pregnant women (Sander *et al.*, 2009). The higher prevalence of malaria in pregnant women than their non-pregnant counterpart (Singh *et al.*, 1999; Nduka *et al.*, 2006) may be the result of the initial lack of immunity against pregnancy associated malaria.

Apart from spontaneous abortion, low birth birth weight is another effect attributable to PAM (Ayoola *et al*., 2012; McClure *et al.*, 2014) as indirect consequences on the foetus from placental and cord blood sequestration of parasite. These effects on the foetus result from interference with placento-foetal exchange, shortage of glucose and oxygen supply to foetus and maternal anaemia (Guyatt and Snow, 2004; Ayoola *et al.*, 2012). Disturbance of foetal growth influencing substances such as placental lactogen, placental folate metabolism and insulin-like growth factor (IGF) by placental sequestration have been suggested (Brabin *et al.*, 2004). Low birth weight is associated with high infant and neonatal mortality. The risk for neonatal mortality increases steadily as the birth weight decreases to below the low birth weight threshold (Guyatt and Snow, 2004).

The transfer of *Plasmodium* from maternal circulation through the placental into foetal circulation is yet to be established, but the possibility of such may not be ruled out by reason of recent research findings. *P. falciparum* isolates from the cord (Ayoola *et al.*, 2012) have been shown to be acquired antenatally by transplacental transmission (Malhotra *et al.*, 2006). This

cord blood localization of parasite presents yet another possibility beyond the indirect intereference with foetal growth and survival. Though not yet proven, *P. falciparum* may directly interfere with the foetus. *P. falciparum* infected erythrocytes (Mantel *et al.*, 2013) and *Plasmodium yoelii* infected reticulocytes (Martin-Jaular *et al.*, 2011) have been shown to exuded extracellular vesicles. Similar vesicles exuded by *Leishmania*-infected macrophages originate from the parasite and carry its molecules to other cells (Silverman *et al.*, 2010). Though not yet reported to the best of my knowledge, it is possible that such vesicles are exuded into the cord environment consequently educating and modifying the foetal immune system. Such communication may dampen or prime the foetal immunity against parasite. Observation in foetal circulation of IgM antiobodies that specifically bind P. *falciparum* (Xi *et al.*, 2003) may suggest foetal-maternal interface antigen exposure if not direct transfer of parasite. In the same vein, *P. falciparum* strains from cord blood different from those in maternal circulation have been reported (Kamwendo *et al.*, 2002). The initial held view that foetal haemoglobin (HbF) inhibits *P. falciparum* growth in the first month of neonatal life has been questioned by a recent finding. Sauerzopf *et al.* (2014) reported that HbF-containing erythrocytes were permissive to *P. falciparum* growth *in vitro* and was not significantly different from that in maternal blood, even though growth rate was reduce by yet unknown factor when cord and maternal plasma was added. This further highlights the need for research into PAM.

Epidemiological assessment of PAM in the malaria endemic regions show higher prevalence in African and Asian countries compared to their American counterpart, irrespective of diagnostic procedure employed (Figure 6). A recent report of 68.1%, 66.7% and 66.6% prevalence of PAM in primigravidae, secondigravidae and multiparous women respectively attending antenatal clinics in Eastern part of Nigeria (Ivoke *et al.*, 2013) which was corroborated by another recent report of 52% overall prevalence of PAM from Western part of same country (Ayoola *et al.*, 2012) remarkably paints the unfortunate picture of sub-Saharan Africa in her fight against maternal and neonatal/infant morbidity and mortality. This is especially deplorable as *P. falciparum* is the endemic species in this area. A much lower prevalence of 1.2% from Chhattisgarh in India between 2007 and 2008 and 13.3% from Sumba in Indonesia between 2008 and 2010 was reported in a comprehensive review by Rijken *et al.* (2012) on PAM in Asian-Pacific region where infection is transmitted by *P. falciparum* and *P. vivax.* This lower

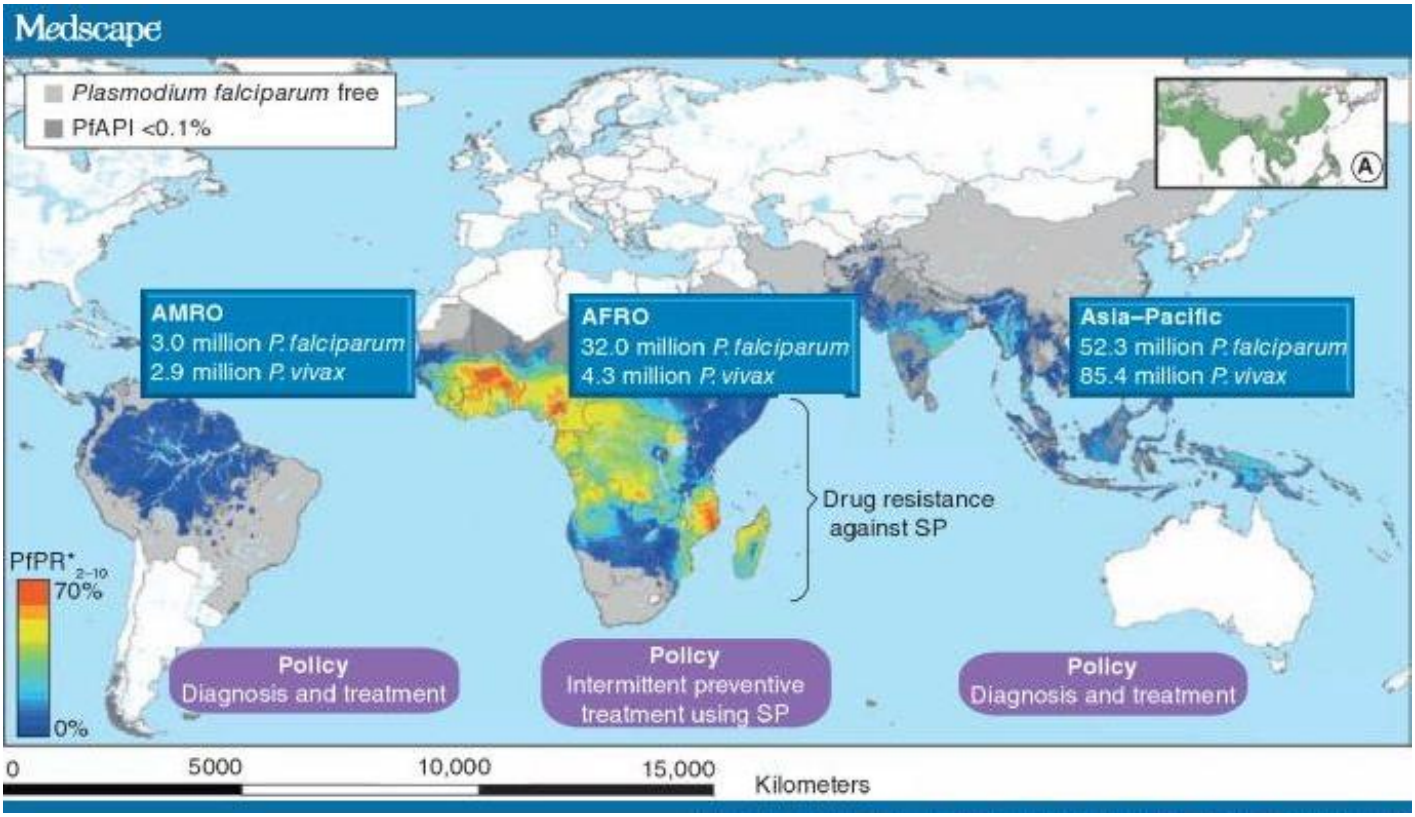

Figure 6: Risk distribution for malaria in pregnancy
Source: Conroy *et al.* (2012)

prevalence does not, however, exclude them from the list of PAM troubled regions. Data on the burden of PAM in Latin America (Central and South America and the Caribbean) is scanty, but CDC (2012a) estimate has it that 4 in every 100 pregnant women suffer PAM.

Appropriate and prompt diagnosis and chemotherapy requiring stringent consideration for duration of pregnancy remains a standard management procedure (WHO, 2015). Chemotherapeutic agents such as chloroquine, Clindamycin, hydroxochloroquine, mefluoquine and artemesinin combination therapy are effective depending on the intensity of virulence. Serious care is recommended in chemotherapy during pregnancy, as teratogenic agents could disrupt foetal development especially at the first trimester. Intermittent preventive treatment with sulphadoxin-pyrimethamine (IPTp-SP) accompanied by the use of ITNs and prompt and effective case management have been recommended. As an update to this recommendation, WHO (2013) advised that 3+ doses of IPTp-SP is more effective than 2 doses. This updated recommendation has been supported by observations that women that had 3+ doses of IPTp-SP delivered babies with better birth weight than those that had less (Walker and Cairns, 2015).

Present research efforts encompass not only the development of appropriate chemotherapeutics but development of both appropriate and cost effective diagnostic procedure especially as most of those afflicted by PAM are in sub-Saharan Africa where poverty is high (Baingana and Bos, 2006). Improving sensitivity and specificity in the detection of sub-microscopic parasitaemia which will facilitate better management of disease before higher and more deleterious parasitaemia develop, remains a major challenge (Maestre and Carmona-Fonseca, 2014). Even though microscopy remain the standard diagnostic method, and RDTs keeps gaining widespread acceptance in malaria diagnosis (Wongsrichanalai *et al.*, 2007; Thiam *et al.*, 2011; Amalu *et al.*, 2012), PCR technique which is bedeviled by demand for high technical expertise and cost has been shown to be more effective in the detection of submicroscopic parasitaemia than the former (Mayengue *et al.*, 2004; Fontecha *et al.*, 2012). An alternative to the detection of parasites or its antigen in the blood is the assessment of plasma or serum biochemical changes (Asaolu and Igbaakin, 2009; Ayoola *et al.*, 2012) which holds promises. This is based on the tendency of the parasitic infection to interfere with the levels of the biochemical in circulation. Development of vaccine against the *var* gene-family product (Khattab *et al.*, 2004; Taylor *et al.*, 2004; Semblat *et*

al., 2006; Magistrado *et al.*, 2011; Clausen *et al.*, 2012) is yet another viable and promising research area targeted at blocking placental adherence of *P. falciparum.*

PAM is also characterized by modification of maternal physiological and biochemical state. Anaemia (Singh *et al.*, 1999), interference with liver activity (Elbadawi *et al.*, 2012), depletion of essential micro- and macro-nutrients (Gibson and Huddle, 1998; Ogbodo *et al.*, 2014) and alteration of plasma lipid and protein profile (Fisayo, 2007; Ayoola *et al.*, 2012; Akanbi, 2013) are some.

1.2.5 Pregnancy-associated malaria and plasma biochemical changes

Plasma biochemical changes have overtime served important diagnostic functions. They are widely used for the assessment of physiological, anatomical, immunological and pathological state of an organism. Physiological and pathological stressors may skew specific biochemical marker away from accepted normal range, or may even instigate introduction into the circulation of non-classical (Anderson and Anderson, 2002) plasma proteins from stressed tissues. Some new exudates from glands may characterize normal but changed physiologic state. Singly and collectively, *Plasmodium* parasites and pregnancy collectively induce alterations in plasma biochemical profile.

As already stated, pregnancy induces changes in plasma lipid and protein profile (Costantine, 2014), and that of other biochemical. As a metabolic adaptation to the demand of pregnancy, such changes may be directed at increasing substance transportation to the foetus from the maternal circulation; protecting the gravida against excessive blood loss at delivery; or suppressing maternal immunologic response to retain foetus. Coagulation and fibrinolytic pathways changes during pregnancy to favour a hypercoagulable state. Plasma levels of clotting factors (VII, VIII, IX, X, XII), fibrinogen and von Willebrand factor increase during pregnancy while factor XI decrease; prothrombin, factor V, protein C and anti-thrombin III remain unchanged (Pacheco *et al.*, 2013). The increase in plasma levels of plasminogen activator inhibitor 1 (PAI-1) and reduced plasminogen activator (RPA) suppress the fibrinolytic system

(Pacheco *et al.*, 2013). This hypercoagulable state is believed to reduce blood loss during delivery (James, 2009). Plasma glucose level also shows remarkable changes during pregnancy. Normal pregnancy is a diabetogenic state characterized by increase in postprandial glucose and associated rise in circulatory glucose insulin response in late gestation (Catalano, 2002). Increased insulin resistance which leads to rise in glucose level is the results of elevation in placental lactogen, oestrogen, progesterone and cortisol in circulation (Pacheco *et al.*, 2013). Adiponectin, leptin, TNF-Ŭ, resistin, IL-6, are other factors that have been implicated in this rise (Barbour *et al.*, 2007). Insulin resistance in type 2 diabetes and obesity is closely associated with intramyocellular lipid. But recent study (Hodson *et al.*, 2013) to ascertain whether insulin resistance in pregnancy show similar association to intramyocellular lipid was to the contrary. The observation showed that while insulin sensitivity decreased during pregnancy, the intramyocellular lipid level was same level during pregnancy and non-pregnancy. The elevations in insulin concentration are more pronounced in lean than obese women (Catanalo, 2002). The pregnancy-associated rise in circulatory glucose facilitates foetal-placental glucose exchange, even though it may predispose the pregnant women to type 2 diabetes (Barbour *et al.*, 2007; Pacheco *et al.*, 2013).

Normal pregnancy is associated with progressive elevation in the levels of cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) (Diareme *et al*., 2009; Al-Tawil, 2013). A similar observation for TC and HDL-C was made by Loke *et al.* (1991). In another study, a significant increase in TG and very low density lipoprotein cholesterol (VLDL-C) and decline in LDL-C level but no statistically significant change in TC and HDL-C levels was reported (De *et al.*, 2006). Elevated lipid levels during early and late pregnancy are associated with complications and adverse outcome for both the gravid and foetus and neonate (Vrijkotte *et al.*, 2012). Non-fasting TG level was observed to be linearly associated with pregnancy-induced hypertension (PIH), preeclampsia and induced preterm delivery. Every increase in non-fasting TG level was linearly associated with rise in PIH, preeclampsia and induced preterm delivery (De *et al*., 2006; Vrijkotte *et al*., 2012). Al-Tawil (2013) reported a significant decline in total protein and albumin levels especially in the second and third trimester of normal pregnancy, whereas the globulin level showed no significant change.

The rise in lipid profile observed during the second and third trimester of normal pregnancy may be associated with increased adipose tissue lipolysis. Other changes maybe the result of transport of lipid from maternal circulation to the foetus. Accumulation of fat in the adipose tissue occur during the first trimester of normal pregnancy while increased adipose tissue lipolysis take place in the last trimester (Diderholm *et al.*, 2005). Increased lipolysis during pregnancy contributes to hyperlipedaemia which provides substrate for energy metabolism in maternal system sparing glucose for the foetus. Intra-uterine growth restriction (IUGR) is probably associated with lower rate of lipolysis or glycerol production. Glycerol production was observed to be lower in women with IUGR than in those with normal pregnancy (Diderholm *et al.,* 2006). Lipoprotein receptors and fatty acid binding proteins in the placenta are known to allow the transfer of long chain polyunsaturated fatty acids (LC-PUFAs) to the foetus during late pregnancy when foetal growth rate is maximal (Herrera *et al.*, 2006). The increase in the plasma levels of VLDL-C, TC, LDL-C and HDL-C in late gestation facilitate the transport of LC-PUFAs in the maternal circulation and by the aid of the lipoprotein receptors in the placenta, allow their uptake by the placenta where they are hydrolysed by lipoprotein lipase, phospholipase A and intracellular lipase to release fatty acids that diffuse into foetal plasma when metabolized (Herrera *et al*., 2006).

Modification of plasma biochemical profile especially lipid profile by malaria has elicited attention as well. Neves *et al.,* (2013) in a cross-sectional study in Anajas a city in Brazil reported a hypocholesterolemic condition that resulted from *Plasmodium* infection. A similar study in another endemic area, Port Harcourt in Nigeria by Chikezie and Okpara (2013) reported lower concentration of HDL-C and LDL-C in malarious than non-malarious individuals. A systematic review and meta-analysis of data from major data bases (Medline/Pubmed, Embase, Cochranes Central Register of Controlled Trials, Web of Science, African Index Medicus, Biosis Previews, and Latin American and Caribbean Health Sciences Literature, LILACS) to determine malaria and febrile diseases associated plasma lipid changes was conducted recently by Visser *et al.* (2013). From their result, TC, HDL-C and LDL-C concentrations were observed to decline during malaria and other febrile diseases with malaria having a significant lowering effect compared to other febrile diseases (Visser *et al.,* 2013). They also observed that TG

concentration increased though it was not significantly different in symptomatic compared to the control.

Even though most recent studies on malaria-associated biochemical changes have focused on TC, TG, HDL-C and LDL-C, Abdagail and ElBagir (2009) in addition to assessing changes in lipid in their study population which were only males, observed changes in the globulin, testosterone and cortisol. They reported significant ($P<0.05$) reduction in total globulin concentration from the heavily and lightly infected patients. Cortisol, a stress hormone, showed a significant increase (191.03±18.17ng/ml) in the heavily-infected compared to the control group (166.28±10.63ng/ml). Modification to the levels of immunoglobulin (Ig) G, IgM, IgA and total protein and albumin in PAM was reported in Nigeria (Fisayo, 2007): a significant ($P<0.05$) decline in IgG, IgA, albumin and total protein was observed, but a slight increase in IgM was noticed. Humoral response is believed to be recruited against erythrocytic stage of malaria parasite. It is therefore expected that IgG and IgM levels increased during malaria infection whether PAM or otherwise (McGregor *et al.*, 1970; Nielsen *et al*. 2005, Mayor *et al*., 2012, 2013). But why Fisayo (2007) noticed a decline is unclear. The modification to maternal peripheral and placental Igs and cytokines in relation to gravidity and trimester of PAM require critical assessment. This is as switch of placental localized (and possibly maternal systemic) immunologic activities to cell-mediated is believed to be associated with the spontaneous abortion in severe PAM cases.

Factors behind malaria-associated changes in plasma/serum biochemical are diverse. Stress to the liver by hepatocytic (pre-erythrocytic) stage of *Plasmodium* sp. may lead to the rise in the level of liver dysfunction enzyme markers, aspartate aminotransferease (AST) and alanine aminotransferase (ALT) (Elnoman Elbadawi *et al.*, 2012; Chikezie and Okpara, 2013). Recruitment and metabolism of biochemicals by parasites is a possible contributor. Biotin-labeled and radio-iodinated polypepetides of molecular sizes ranging from 45 to 206kDa were internalized irrespective of their primary sequence by *P. falciparum in vitro* (El Tahir *et al.*, 2003). Human serum albumin which is important to *P. falciparum* development was observed imported into the parasite in the same study (El Tahir *et al.*, 2003). Most protozoan parasites either completely lack or have incomplete *de novo* lipid synthesis (Rub *et al.*, 2013).

Plasmodium cannot synthesize fatty-acid and cholesterol *de novo* and thus meets their demand for these substances by recruitment from host cell (e.g hepatocytes by pre-erythrocytic stage) (Labaied *et al.*, 2011). HDL-C is important for *P. falciparum* but only within a specified range above which it becomes harmful to the parasite (Bansal *et al.*, 2005).

Parasites may alter the human plasma environment by their secretory products. *P. falciparum* is known to export a number of proteins such as PfEMP-2, *P. falciparum* histidine rich protein (PfHRP)-1, and PfHRP-2 into host cell and extracellular milieu (El Tahir, 2003). Release from *P. falciparum*-infected intra-erythrocyte environment of extracellular vesicles laden with *P. falciparum* parasite molecules (Mantle *et al*., 2013) is another interesting observation. These vesicles have also been demonstrated to be exuded by *Leishmania donovani* within a macrophage and were transported from the macrophage cytosol through the extracellular milieu into neighbouring macrophages (Silverman *et al.*, 2010). Enzymes such as phospholipase, acytransferase, sphingomyelinases and sphingolipid synthases that target host lipidome performing functions which include cell penetration and triggering of signaling pathways (Rub *et al.*, 2013). Parasites may also release certain lipids (e.g eicosanoids and ceramides) intracellularly and extracellularly to modulate selfishly immunologic activities of the host. The vast array of substances released by parasites may find their way into the plasma as foreign biochemical. They exact influence on disease outcome as they may modulate immunologic response in parasite favour, facilitate parasite establishment and proliferation, or define disease pathogenesis and the associated damages to the host.

The combined effect of pregnancy and malaria (PAM) on the plasma biochemical is presently gaining attention among researchers as a result of the high maternal and neonatal/infant mortality and morbidity associated with PAM. In areas of stable malaria risk in sub-Saharan Africa, PAM is estimated to cause 100 000 annual infant mortality (Guyatt and Snow, 2004). In malaria endemic areas, it is estimated to be associated with LBW (8 ï 14%), preterm-LBW (8 ï 36%), IUGR-LBW (13 ï 70%) and infant mortality (3 ï 8%) (Steketee *et al.*, 2001). In sub-Saharan Africa alone, approximately 25 million pregnant women are at risk of *P. falciparum* infection (Desai *et al.*, 2007).

Ayoola *et al.* (2012) observed a significantly lower TC, HDL-C and LDL-C, but higher Tumor necrosis factor (TNF) in both the second and third trimesters, but there were no significant changes in TG, glucose and insulin levels for either trimesters. Maternal TC and LDL-C were significantly correlated with birth weight (P<0.001) and insulin (0.001) while maternal TG and glucose and cord blood insulin and IGF-1 correlated with birth weight (P<0.05) (Ayoola *et al.*, 2012). There is, however, paucity of research on PAM and associated maternal lipid changes. While recent researches focusing on placental malaria consider plasma protein and receptors, attention is mainly focused on antibody against placental *P. falciparum* strains of VAR2CSA.

1.2.6 BMI and plasma biochemical changes in pregnancy-associated malaria

Pre-pregnancy and pregnancy weight or BMI contributes to pregnancy outcome. Pre-pregnancy body mass and weight gain during pregnancy are important measures of maternal nutrition, a major determinant of neonatal survival, neonatal BW and childhood and adulthood height, health and cognitive ability (Adair, 2007; Almond and Currie, 2011; Ludwig *et al.*, 2013, Coffey, 2015). The relatively shorter and smaller Indian children compared to their sub-Saharan African counterpart has very recently been attributed possibly to the higher percentage (42.2%) underweight pre-pregnant women in India compared to the 16.5% in sub-Saharan Africa (Coffey, 2015). This problem is further complicated by yet another estimate that places weight gain at full-term pregnancy in both regions at only about 7kg which is much lower than the 12.5 to 18.0kg recommended for underweight pre-pregnancy and almost completely out of that recommended for overweight (7.0 ï 11.5kg) as well (Institute of Medicine [IOM], 2009; Rasmussan *et al.*, 2009; Coffey, 2015).

Weight gain or reduction induces plasma lipid changes. A meta-analysis by Dattilo and Kris-Etherton (1992) reported that weight reduction was associated with significant decrease (PÒ 0.001) and correlations (PÒ 0.05) for TC (r = 0.32), LDL-C (r = 0.29), VLDL-C (r = 0.38) and TG (r = 0.32). From this analysis of 70 studies on the effect of dieting on plasma lipid changes, Dattilo and Kris-Etherton (1992) observed a 0.09mmol/L increase (PÒ 0.01) in HDL-C for subjects at a stabilized, weight reduction and 0.07mmol/L decrease (PÒ 0.05) in those actively

losing weight. Their result was, however, independent of pregnancy and sex. Another research on weight loss in postmenopausal women reported 11.4%, 15% and 18% reduction in TC, TG and LDL for 9.4% change in BMI from baseline of 35.95±5.32 in a duration of 6months (Cordero-MacIntyre *et al.*, 2004). This two preceding reports (Dattilo and Kris-Etherton, 1992; Cordero-MacIntyre *et al.*, 2004) taking together with other reports (Adair, 2007; Ludwig *et al*., 2013; Coffey, 2015) implies that specific lipid changes may act as predictors of not just the immediate pregnancy outcome as it concerns the neonate but his/her life thereafter. This suggestion when considered with reference to the significant decline in HDL-C, TC, LDL-C in PAM cases in Nigeria (Ayoola *et al.*, 2012) may present remarkable outcomes.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Study Area

Igbo-Eze North, a Local Government Area in Enugu State, Nigeria, is located between latitude 7º00ɑand 7º12ɑN and longitude 7º12ɑand 7º36ɑE (Cartographic Unit, Department of Geography, 2015) (Figure 7, 8). It has a land area of 293km^2 and bordered by Kogi to the northwest, Benue to the northeast, Anambra to the west, Abia to the south and Ebonyi to the east (Enugu State Official Website, 2015; Cartographical Unit Department of Geography, 2015). Enugu state is a tropical Guinea Savanna. It is characterized by two seasons just like other West African countries: the rainy and dry season. The rainy season commences in April and retreat in October; dry season commence in November (Igwenagu, 2015). The mean daily temperature is 26.7ºC (80.1ºF) and average annual rainfall 2000 millimetre (66.73 inch) (Igwenagu, 2015).

Enugu-Ezike where the District Hospital is situated is the Local Government Headquarter of Igbo-Eze North Local Government Area. Enugu-Ezike is a semi-urban settlement. Other communities in Igbo-Eze North Local Government Area include: Ette, Olido, Igogoro, Aji, Imufu, Umuogbu-Agu, Agu-Ibeje, Ikpamodo, Umuopu-Agu, Ufodo, Ikpiga, Ukwuinyi, Amachara, Ugbaike and Umuagama, Onicha-Enugu, Amufe, Nkpamute, Ogrute, Umuida, Umuogbo-inyi, Udah and Okata (Figure 8). Some of these communities are semi-urban and others villages. The people who are Igbo speaking are mainly farmers and traders. The people in the villages and several residences in the semi-urban areas are in close proximity to bushes that serve mainly for agricultural purpose (Figure 9). Some plants grown in these areas include oranges, pawpaw, cashews, mango, banana, pineapples, oil palm, melon, pumpkins, pepper, kolanuts, cashew, maize, yam, cocoyam and groundnut (Omeje and Ajayi, 2009; Chah and Igbokwe, 2011).

Health care provision in Igbo-Eze North Local Government Area is disparate. Public primary, public secondary and private health facilities are distributed around the Local Government Area. The health facilities include District Hospital Enugu-Ezike, Aguibeje Health Centre, Aji Health Centre, Ette Ulo Health Centre, Igogoro Health, Inyi Health Centre, Ikpamodo Health Centre Health, Imufu Health Centre, Olido Health Centre, Ette Cottage Hospital, Ufodo Health Post and Umuagama Health Post (Referral Directory, 2011). From preliminary investigations before the

commencement of this study, it was discovered that some of the health centres were non-functional while some were poorly furnished for antenatal purpose. Some, however, such as the District Hospital where this study was carried out was among the well furnished and staffed hospitals in the Local Government Area. This excellent status of the District Hospital Enugu-Ezike endeared it to several pregnant women.

According to the 2006 National Census figures, Igbo-Eze North has a population size of 258, 829 which is about 8.0% of Enugu State population placed at 3, 267,837 with an annual growth rate of 3.0% (National Population Commission [NPC], 2010b). Females constitute 51.1% of Enugu State population and women of reproductive age (15 ï 49 years) was put at 908,985 (and 69,683 in Igbo-Eze North) with an annual fertility ratio of 4.8 (NPC, 2010a,b; NPC and ICF, 2014). Maternal mortality ratio (MMR) in Enugu State is high, placed at 1,400 per 100,000 live births according to the Nigeria Demographic and Health Survey (NDHS) of 2003 (NPC and ORC Macro, 2004). This high MMR has been attributed to both medical and socio-cultural factors which include low doctor to pregnant women ratio (1:1,581), obstetric deaths, preventable pregnancy complications and poor utilization of antenatal care services due mainly to poverty, ignorance and inadequate health facilities (Okeibunor *et al.*, 2010). The 2013 and most recent NDHS places neonatal, postneonatal and infant mortality in the South East of Nigeria where Enugu is situated at 37, 45 and 82 per 1000 live births respectively (NPC and ICF, 2014).

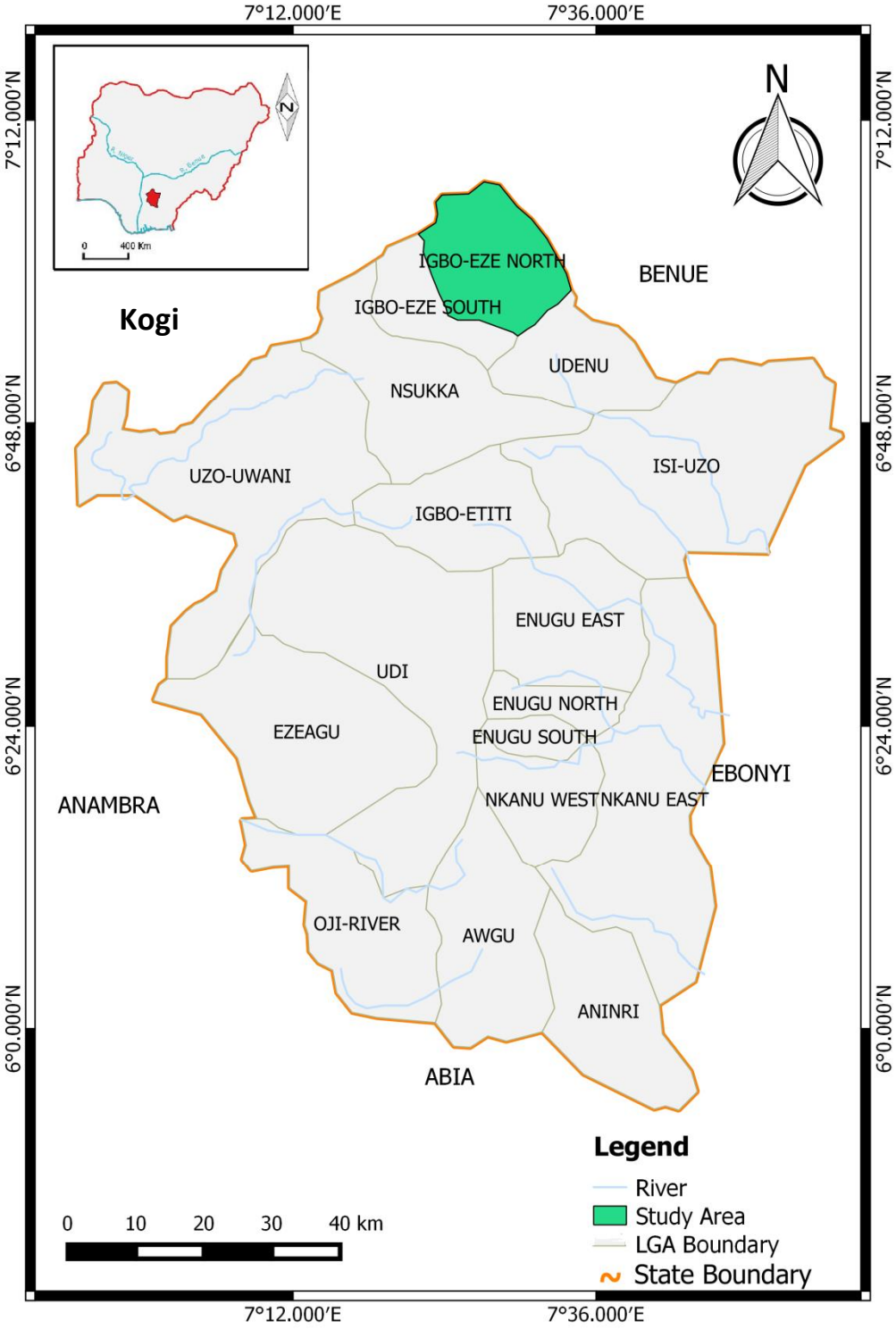

Figure 7: Map showing Igbo-Eze North Local Government Area
Source: Cartographical Unit Department of Geography (2015)

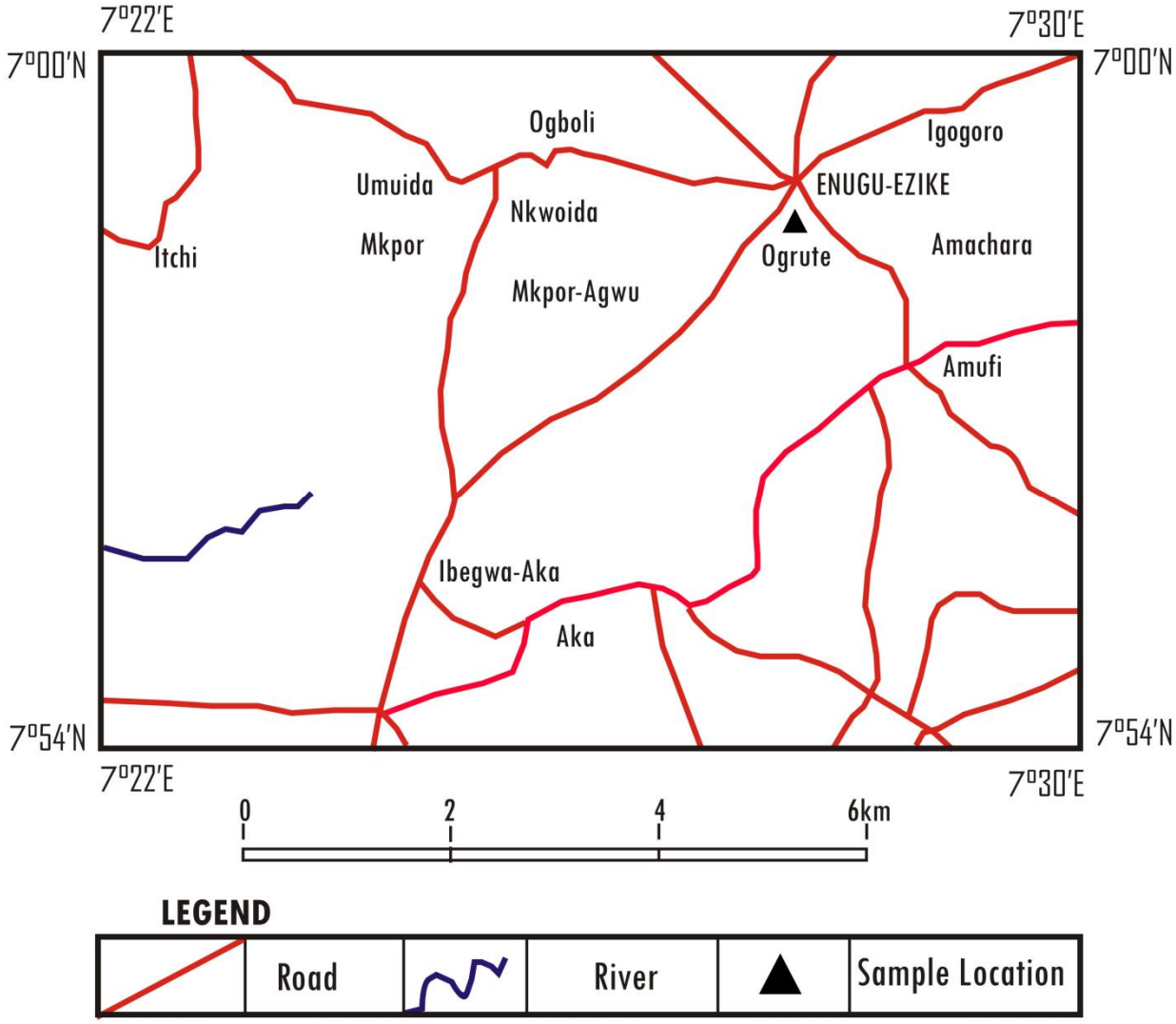

Figure 8: Map of study area showing sampling site

Source: Adapted from Google Map (2015)

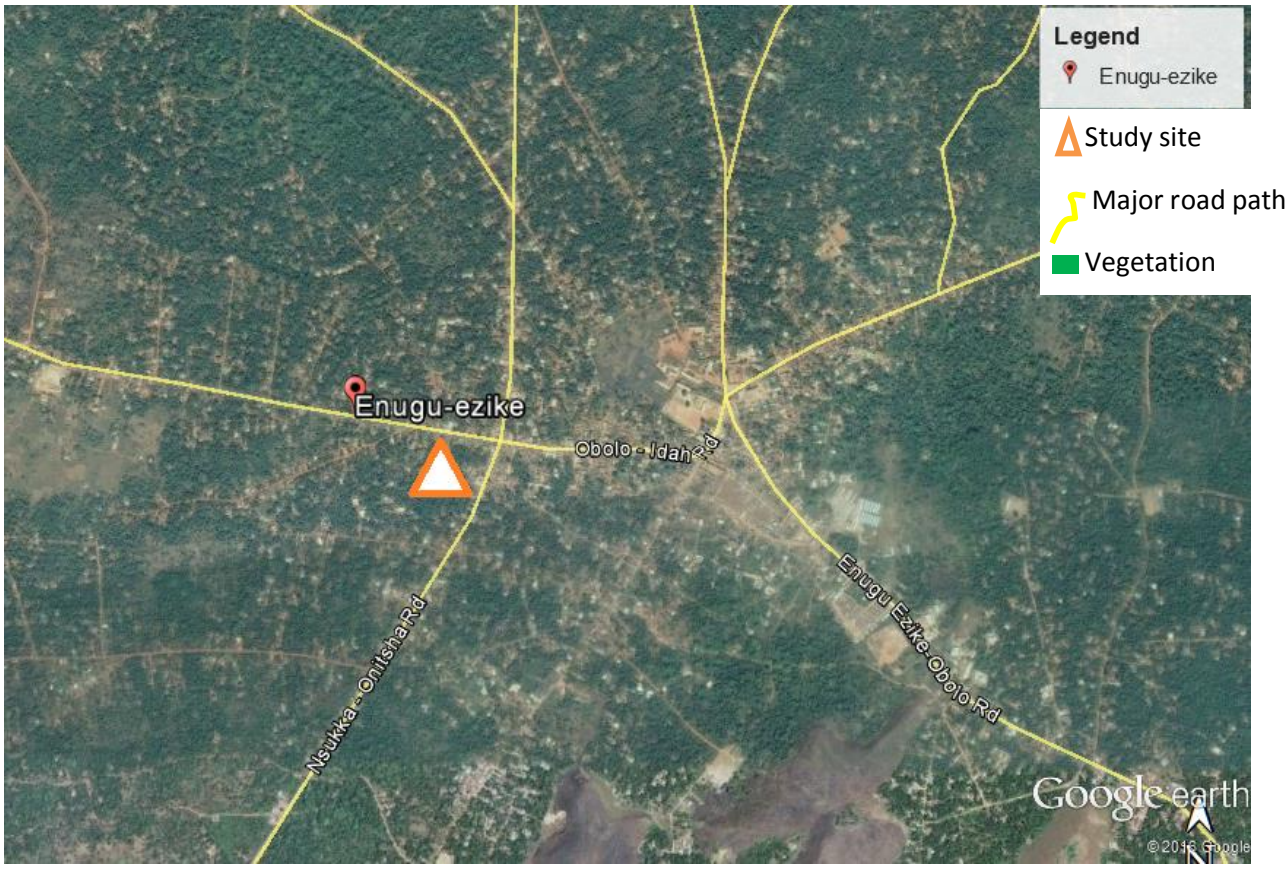

Figure 9: Google image of study area showing study site and surrounding vegetations in green patches
Source: Google Map (2016)

2.2 Study Design

A cross-sectional study design was used to investigate the plasma lipid, serum protein and body mass indices among women attending antenatal clinics in Igbo-Eze North Local Government Area, Enugu State, Nigeria.

2.3 Ethical Clearance

The study protocol was approved by the ethical committee of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria. Ethical clearance was also obtained from the University of Nigeria Teaching Hospital (UNTH) Enugu State, Nigeria (Appendix, A). Approval was obtained from the Department of Health Enugu Ezike Local Government Area secretariat (Appendix, B). Blood samples and BMI data were collected at the consent of subjects (Appendix, C). Informed approval was obtained from the Chief Medical Officer at the District Hospital, Enugu Ezike for the conduct of the study in the hospital.

2.4 Sample Size Selection

After feasibility study and familiarization visits to the study area, the District Hospital Enugu-Ezike was considered the most suitable place for this study. Several reasons informed the choice to restrict the work to this hospital. The health clinics in some communities were non-functional. Those functional were poorly staffed and attended very little to pregnant women. Majority of the pregnant women prefer to attend quack private clinics rather than attend the local health centres. Several pregnant women, however, come from the villages to the District Hospital Enugu-Ezike. The District Hospital was well furnished and staffed.

Sample size was determined using the formula:

$$N = \frac{(S_{\sigma})^2 Z^2}{B^2} \quad \text{(McClave and Benson, 1991)}$$

where,

N= estimated sample size

σ^2 = population standard deviation ~ S, sample standard deviation

B = width of deviation (i.e. margin of error)

Ŭ= level of significance taken as 0.05 (i.e 95% confidence level).

$Z_{Ŭ2}$ = critical value

From the record of antenatal attendance at the District Hospital obtained from Health Management Information System with the assistance of the Local Government Health Information Expert, Mr Oshomi Vincent, the sample size was estimated (Appendix C). The total six monthly (July to December) antenatal attendances for 2012, 2013 and 2014 were initially used. The 2015 recorded was also verified in January 2016 to re-assess the reliability of our estimate. The estimated monthly attendance for the three years was appromimately 12. Thus the estimated sample size for the 4 months duration of sample collection was 48. Another estimate was made using online sample size calculator software which obtained an estimate of 84 (at 20% margin of error) (FluidSurveys, 2015). Thereafter, sample size of 48 to 80 was considered a reliable estimated sample size for the population antenatal visitors to the District Hospital. A sample size of 75 was chosen as a reliable estimate.

Another estimate in January 2016 using data for 2012 to 2015 placed the estimate at 61 when the equation shown above was used, while the online calculation remained unchanged. Considering the report of over 10% monthly repeated attendance; and the assumption that over 10% attended at least twice in a month. Also considering the report of less than 10% delivery at the hospital compared to total attendance, the margin of error of 20% was considered reliable. The total number of pregnant women that reported for antenatal for duration of the study did not exceed 90.

2.5 Anthropometric Measurements

Anthropometric data, weight and height were measured using a beam balance scale (to the nearest 0.5kg) and standiometer (to the nearest 0.1cm) respectively, in line with internationally accepted standard techniques (Tolonen *et al.*, 2002a). Body mass index (BMI) was calculated using the formula:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \qquad \text{(Keys } et\ al.,\ 2014\text{)}$$

BMI below 18.5 was considered underweight, 18.5 ï 24.9 as normal, 25.0 ï 29.9 as overweight and 30.0 and above as obese.

2.6 Questionnaires

The Questionnaire (Appendix, D) which was test ran for this study purpose was designed to significantly reduce time expended and stress. This was done by making the question concise and admistering them verbally by the researcher with the subjects sitting. The questions asked covered maternal age, gravidity, parity, and duration of pregnancy. Questions such as community of residence, ownership and usage of mosquito bednet and use of intermittent preventive therapy for malaria were only included as assistance for comprehension of pregnancy-associated malaria predisposing factors in the studied population. The other questions served as criteria for inclusion and exclusion. Those that answered óyesô for the following questions were excluded from the plasma lipid assay tests: fasting, diabetic, liver diseases, alcohol intake, smoking, on weight regulating chemotherapy, strenuous exercise, and to suffering other infectious/parasitic diseases at the time of study were excluded. Those whose hospital record showed that they suffered from pre-pregnancy high blood pressure were excluded as well.

2.7 Collection of Blood Samples

For malaria diagnosis, plasma lipid and serum protein assays, from the anticubital fossa 3 ml of venous blood were collected by normal process of venepuncture with subject in a sitting position. The subject was made to sit for at least 5 minutes before blood was collected. Tourniquet was released within 1 minute of application before blood collection. Blood collection and handling procedure were in compliance with recognized standard practices (Tolonen *et al.*, 2002, 2002b; Deeg, 2006; WHO, 2007; National Health and Nutrition Examination Survey [NHANES], 2008, 2009, 2010; Craig *et al.*, 2013). Blood samples were collected by a qualified nurse well-trained in venepuncture.

2.8 Malaria Diagnosis

Four drops of the collected blood was immediately used for malaria diagnosis. Thin and thick blood smears for malaria parasites were prepared and stained with 3% Giemsa at pH 7.2. Blood films were examined using light microscope and recorded as negative only after 100x, oil

immersion was used to count > 1900 RBCs in thin smears. *Plasmodium falciparum* in blood sample was specifically identified by presence of one or more of these conditions: multiple infection of RBCs, high parasitaemia, presence of one or two chromatin dots, presence of Maurerôs cleft, crescent-shaped gametocytes, occasional appliqué forms and schizonts where present, with upto 30 merozoites (CDC, 2013). Malaria parasite density was determined using the formula:

$$\frac{\text{Number of parasites counted}}{(\text{number of WBCs counted})} = 5.0 \times 10^{3} \times \text{number of parasites counted per 2000 WBCs}$$

Where,

RBC = red blood cell (Research Malaria Microscopy Working Group, 2015).

Prevalence was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Number of individuals with malaria parasites}}{\text{Total number of individuals examined}}$$

The assistance of a trained microscopist was employed as recommended by WHO, (2000).

Thick and thin blood smear was prepared in accordance to procedure by CDC (2013) as follows:

1. Pre-cleaned slides were labelled;
2. Slides were cleaned with 70% ethanol and allowed to dry. Slides were held in such a way as to avoid finger contact with the surfaces;
3. Site for blood collection was cleaned using 70% alcohol and allowed to dry;
4. For thin blood smear,
    a. A small drop of blood was placed on the slide towards one end;
    b. A clean spreader was held at a 45º angle to the drop of blood on the specimen slide and in contact with it;
    c. The blood was allowed to spread along the entire width of the spreader slide;
    d. While holding the spreader slide at the same angle of 45º, it was pushed forward rapidly towards the other end of the slide;
    e. The thin smear of blood was allowed to dry.
5. For the thick smear,
    a. A drop of blood was placed on a clean slide;

b. The drop of blood was spread to obtain a diameter of 1 ï 2cm. The smear was such that one could read a newsprint through itô
c. The thick smear was allowed to air dry before fixing.

6. Before staining, the thin smear was fixed with absolute ethanol
7. Giemsa (3.0%) was used to dropped to cover each smear with the slides placed on a staining rack;
8. After 30 minutes, each slide was gently rinsed in a pool of water and allowed to air dry in a slanting position.

2.9 Biochemical Assays

One ml of blood was transferred into a plain vial for serum protein assays, and the remaining 2ml into a heparinized vial for plasma lipid assays. Biochemical assays for lipids and protein is as outlined below.

2.9.1 Plasma lipid assays

TC, TG, HDL-C were assayed by a combination of enzymatic and spectrophotometric methods using a manual analyzer, Spectrolab 23A. LDL-C was estimated using Friedewald formula:

[LDL-C] = [TC] ï [HDL-C] ï [TG]/5

Where,

LDL-C = low-density lipoprotein cholesterol
HDL-C = high-density lipoprotein cholesterol
TG = triglyceride
TC = total cholesterol (Friedewald *et al.*, 1972).

Randox Diagnostic Kit (Randox Laboratories Ltd., United Kingdom) were used for the direct assays for TC, HDL-C and TG and recommended protocol for manual analysis as outlined below was strickly adhered to:

Total Cholesterol (Allain *et al.*, 1974; Randox Laboratories, 2002)

1. Into a clean vacuum tube, Test Reagents, Test Samples, Reagent Standard and distilled water were pipetted as follows:
   Reagent Blank = 10 µL distlled H_2O + 1000 µL Test Reagent
   Standard = 10 µL Reagent Standard + 1000 µL Test Reagent
   Sample (Blood Plasma) = 10 µL Plasma sample (test sample) + 1000 µL Test Reagent
2. The Reagent Blank, Standard and Sample were mixed gently for 5 seconds and incubated for 5 minutes at 37 ºC;
3. Each Sample after incubation was transferred into a curvette before reading was taken using a spectrophotometer;
4. The spectrophotometer was first blanked using the Reagent Blank, after which reading for the Standard ($\phi A_{standard}$) followed by the Samples (ϕA_{sample}) in the order they were prepared. Readings were taken at 500 nm wavelength;
5. All reading were taken in less than 60 minutes post incubation;
6. Concentration of cholesterol in Sample was calculated from the formula:

$$\Delta \_{\_\_\_\_\_} \_{\Delta \_{\_\_\_\_\_\_\_\_\_}} \times \_\_\_\_\_ \_\_ \_\_\_\_\_$$

Concentration of Standard was given by manufacturer as 208 mg/dL or 5.40 mmol/L.

High Density Lipoprotein Cholesterol (Randox Laboratories, 2007a)

1. 1. Into a clean vacuum tube, Test Reagents, Test Samples and Reagent Standard and distilled water were pipette as follow:
   Standard = 200 µL Reagent Standard + 500 µL Test Reagent
   Sample (Blood Plasma) = 200 µL Plasma sample (test sample) + 500 µL Test Reagent
2. The Reagent Blank, Standard and Sample were mixed gently for 5 seconds and allowed tp stand for 10 minutes at room temperature;
3. At the end of 10 minutes, the content of the test tubes were centrifuged for 10 minutes at 4000 rpm;
4. The clear supernatant is immediately separated off for further analysis,
5. The CHOD-PAP method which was the same procedure as that of the TC except for concentration difference was employed as recommended:
   Reagen Blank = 100 µL distlled H_2O + 1000 µL Test Reagent

Standard = 100 µL Reagent Standard + 1000 µL Test Reagent

Sample (Blood Plasma) = 100 µL Plasma sample (test sample) + 1000 µL Test Reagent

6. Each Sample after incubation was transferred into a curvette before reading was taken using a spectrophotometer;
7. The spectrophotometer was first blanked using the Reagent Blank, after which reading for the Standard ($\phi A_{standard}$) followed by the Samples (ϕA_{sample}) in the order they were prepared. Readings were taken at 500nm wavelength;
8. All reading were taken less than 60 minutes post incubation;
9. Concentration of cholesterol in Sample was calculated from the formula:

$$\frac{\Delta \phi_{sample}}{\Delta \phi_{standard}} \times \text{Concentration of Standard}$$

10. Concentration of Standard was given by manufacturer as 208 mg/dL or 5.40 mmol/L.

Triglyceride (Randox Laboratories, 1997)

1. Into a clean vacuum tube, Test Reagents, Test Samples, Reagent Standard and distilled water were pipetted as follows:

   Reagent Blank = 1000 µL Test Reagent

   Standard = 10 µL Reagent Standard + 1000 µL Test Reagent

   Sample (Blood Plasma) = 10 µL Plasma sample (test sample) + 1000 µL Test Reagent
2. The Reagent Blank, Standard and Sample were mixed gently for 5 seconds and incubated for 5 minutes at 37 ºC;
3. Each Sample after incubation was transferred into a curvette before reading was taken using a spectrophotometer;
4. The spectrophotometer was first blanked using the Reagent Blank, after which reading for the Standard ($\phi A_{standard}$) followed by the Samples (ϕA_{sample}) in the order they were prepared. Readings were taken at 500 nm wavelength;
5. All reading were taken in less than 60 minutes post incubation;
6. Concentration of cholesterol in Sample was calculated from the formula:

$$\frac{\Delta \phi_{sample}}{\Delta \phi_{standard}} \times \text{Concentration of Standard}$$

Concentration of Standard was given by manufacturer as 192 mg/dL or 2.17 mmol/L.

2.9.2 Serum protein assays

Blood collected into vial was allowed to stand at room temperature for 2 hours to clot. The clotted blood was centrifuged for 10 minutes at 12 000 rpm. Total serum protein (TP) and Albumin (Alb) assays were done using Randox Diagnostic Kit (Randox Laboratories Ltd., United Kingdom). Globulin was estimated using the formula:

[illegible] – [illegible] = [illegible] (Kinsley, 1939)

Albumin to globulin ratio (A/G) was calculated using the formula:

$$A/G = \frac{[\text{illegible}]\ (g/dL)}{[\text{illegible}]\ (g/dL)}$$

The protein assay protocol was as follow:

Total Protein (Randox Laboratories, 2007b)

1. Into a clean vacuum tube, Test Reagents, Test Samples, Reagent Standard and distilled water were pipetted as follows:
   Reagent Blank = 20 µL distlled H_2O + 1000 µL Test Reagent
   Standard = 20 µL Reagent Standard + 1000 µL Test Reagent
   Sample (Blood serum) = 20 µL Serum sample (test sample) + 1000 µL Test Reagent
2. The Reagent Blank, Standard and Sample were mixed gently for 5 seconds and incubated for 5 minutes at 37 ºC;
3. Each Sample after incubation was transferred into a curvette before reading was taken using a spectrophotometer;
4. The spectrophotometer was first blanked using the Reagent Blank, after which reading for the Standard ($\phi A_{standard}$) followed by the Samples (ϕA_{sample}) in the order they were prepared. Readings were taken at 546 to 600 nm wavelength;
5. All reading were taken less than 60 minutes post incubation;
6. Concentration of cholesterol in Sample was calculated from the formula:

$$\Delta A_{[\text{illegible}]} \div \Delta A_{[\text{illegible}]} \times [\text{illegible}]$$

Concentration of Standard was given by manufacturer as 60 g/L.

Albumin (Randox Laboratories, 2008)

1. Into a clean vacuum tube, Test Reagents, Test Samples, Reagent Standard and distilled water were pipetted as follows:
   Reagent Blank = 10 µL distlled H_2O + 3000 µL Test Reagent
   Standard = 10 µL Reagent Standard + 3000 µL Test Reagent
   Sample (Blood serum) = 10 µL Serum sample (test sample) + 3000 µL Test Reagent
2. The Reagent Blank, Standard and Sample were mixed gently for 5 seconds and incubated for 5 minutes at +20 ºC to 25 ºC (room temperature);
3. Each Sample after incubation was transferred into a curvette before reading was taken using a spectrophotometer;
4. The spectrophotometer was first blanked using the Reagent Blank, after which reading for the Standard ($\varphi A_{standard}$) followed by the Samples (φA_{sample}) in the order they were prepared. Readings were taken at 546 to 600 nm wavelength;
5. All reading were taken less than 60 minutes post incubation;
6. Concentration of cholesterol in Sample was calculated from the formula:

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Concentration of Standard}$$

Concentration of Standard was given by manufacturer as 44.7 g/L.

2.10 Statistical Analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, Illinois) and Microsoft Office Excel (Microsoft Inc.). Data was carefully entered and cross-examined twice before analysis to avoid errors. Tests for normality for plasma lipid, serum protein, albumin, albumin to globulin ratio (A/G), age and BMI were first conducted using Shapiro-Wilk and Kolmogorov-Smirnov tests. Data from subsequent analysis were represented as mean ± SE and percentage (%). Chi-square test was used to compare malaria prevalence and mosquito bednet ownership and usage. Student t/Mann Whitney U Tests were employed in the comparison of maternal biochemical characteristics, age and BMI between trimesters of pregnancy, and between malaria-infected and uninfected women. One way analysis of variance

(ANOVA) and Kruskal-Wallis Test were used to compare maternal biochemical characteristics, age and BMI by gravidity, parity, age and BMI categories. Duncan Multiple Range Test and Bonferroni Test were Post Hoc Tests employed where necessary. Correlation and simple linear regression were used to ascertain the level and nature of relationship between maternal biochemical characteristics, age and BMI and malaria parasite density. All tests were two-sided and $p < 0.05$ was considered significant.

CHAPTER THREE

RESULTS

3.1 Overall Maternal Obstetrics Characteristics

A total of 75 pregnant women attending antenatal clinics in Igbo-Eze North District Hospital, Enugu-Ezike consented to this study. Seventy-two (72) out of these women qualified for plasma lipid and serum protein assays after screening in compliance with the inclusion criteria of the study. The 72 persons for lipid assays were not exactly the same subjects for the protein assays. The only difference in the number of people that qualified for the lipid assays and the protein assays is that the three persons excluded for protein assays were different from those excluded for lipid assay from the same 75 volunteers. Malaria and body mass index (BMI) status of the 75 women were, however, determined.

Twenty-one out of the 75 (28.0%) women were primigravid, 14 (18.7%) secundigravid and 40 (53.3%) multigravid (Table 1). Twenty-six out of the 75 (34.7%) were primiparous, 10 (13.3%) secundiparous, 39 (52.0%) multiparous. Two (2.7%) were at first trimester of pregnancy, 34 (45.3%) at second trimester and 39 (52.0%) at third trimester. Forty-three (57.3%) of the 75 pregnant women were with 20 ï 29 year and 32 (53.4%) within 30 ï 39 years. Twenty-two (29.3%) were within the BMI category 18.5 ï 24.9, 34 (45.3%) within 25.0 ï 29.9 BMI category and 19 (25.3%) in > 30 BMI category. The mean (±standard error) concentration of Total Cholesterol (TC) in the pregnant women was 164.12 ± 4.26mg/dl, the High Density Lipoprotein Cholesterol (HDL-C) 65.98 ± 2.54mg/dl, Triglyceride (Trig) 159.12 ± 8.90mg/dl, and Low Density Lipoprotein Cholesterol (LDL-C) 66.70 ± 4.22mg/dl. The overall prevalence of *falciparum*- malaria was 50.7% as 38 out of the 75 pregnant women examined were infected. The overall mean parasite density per micro litre of red blood cell (RBC) examined was 112894.74 ± 22901.73 (10 000 ï 430 000).

Table 1: Overall maternal clinical characteristics

Lipid (n = 72)		**Protein (n = 72)**	
TC (mg/dl)	164.12 ± 4.26	TP (g/dl)	6.18 ± 0.15
HDL-C (mg/dl)	65.98 ± 2.54	Albumin (g/dl)	3.34 ± 0.07
TG (mg/dl)	159.12 ± 8.90	Globulin (g/dl)	2.85 ± 0.14
LDL-C (mg/dl)	66.70 ± 4.22	Albumin/Globulin	1.55 ± 0.12
(n = 75)			
Age (Year)		BMI (Kg/m^2)	
27.60 ± 0.71 (18 - 38)		27.65 ± 0.52 (20.1 - 41.9)	
***falciparum* Malaria**			
No. Examined (%)	No. Infected (%)	Density (/µL)x10^3	
75 (100)	38 (50.7)	112.89 ± 22.90 (10 - 430)	
Obstetric characteristics			
Gravidity	**No. examined (%)**	**Parity**	**No. examined (%)**
Primigravidae	21 (28.0)	Nulliparity	26 (34.7)
Secundigravidae	14 (18.7)	Primiparity	10 (13.3)
Multigravidae	40 (53.3)	Multiparity	39 (52.0)
Total	75 (100)	Total	75 (100)
Trimesters	**No. examined (%)**	**Age group (Year)**	**No. examined (%)**
1^{st} trimester	2 (2.7)	< 20 ï 29	43 (57.3)
2^{nd} trimester	34 (45.3)	30 ï 39	32 (42.7)
3^{rd} trimester	39 (52.0)	Total	75 (100)
Total	73 (100)		
BMI Categories (Kg/m^2)			
18.5 ï 24.9	22 (29.3)		
25.0 ï 29.9	34 (45.3)		
Ó30	19 (25.3)		
Total	75 (100)		

n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

3. 2 Prevalence and Density of *falciparum* Malaria among Women Attending Antenatal Clinics in Igbo-Eze North Local Government Area

3.2.1 Prevalence and density of pregnancy-associated malaria by maternal obstetrics characteristics

The prevalence and density of malaria parasite by maternal obstetrics characteristics is shown in table 2. The multigravidae had 57.5% prevalence of malaria and contributed the highest percentage (60.5) to the 50.7% overall prevalence when compared to the primigravidae and secundigravidae but this difference was not significant statistically (χ^2 = 2.078, p = 0.354). Though the primigravidae had the least parasite prevalence (38.1%) compared to that among either the secundigravidae (50.0%) or multigravidae (57.5%), they had significantly higher (p < 0.05) parasite density (Figure 10).

Pregnancy-associated malaria cases were higher among the multiparous women compared to the nulliparous and primiparous women. This observed difference was not significant at p < 0.05. A comparison of the parasite density by parity showed that parasite density in the nulliparity group was significantly higher than that of the primiparity and multiparity group (p = 0.002) (Figure 10). The nulliparous women had much higher parasite density than the primiparous (p = 0.022) and the multiparous (p = 0.003) from Bonferroni Post Hoc Test.

A combination of the nulliparous and primiparous women into a new category, the nulli+primiparous, and the primigravidae and secundigravidae to primi+secungravidae also showed significant differences in parasite density when compared to the multiparous and multigravidae respectively (figure 11). The parasite density in the primi+secundigravidae was significantly higher than that in the multigravidae (F = 13.431, p = 0.021). Similarly, the parasite density in the nulli+primiparity group was significantly higher than that in the multiparity group (F = 12.189, p = 0.032).

Still on table 2, when the women were grouped based on the duration of pregnancy in trimesters, the total number of women became 73 as two of them that were in their first trimester were

excluded due to low sample size. Prevalence of pregnancy-associated malaria was 59.0% in the 3rd trimester which contributed 63.9% of the 49.3% overall prevalence for the 73 women. Parasite density was not significantly dependent on trimester of pregnancy (figure 12).

When the pregnant women were regrouped based on age, into those in age group < 20 ï 29 and 30 ï 39, malaria prevalence was observed not to be age dependent in the studied population (ɟ > 0.05); but parasite density was (F = 28.510, ɟ = 0.020) (figure 12).

Table 2: Prevalence and Density of *falciparum* Malaria by maternal obstetrics characteristics

	No. Examined (%)	No. infected (%)*		Parasite
Gravidity		Within gravidity	Within Malaria	Density (/µL)x10^3
Primigravidae	21 (28.0)	8 (38.1)	(21.1)	268.13 ± 58.23
Secundigravidae	14 (18.7)	7 (50.0)	(18.4)	92.14 ± 4.72
Multigravidae	40 (53.3)	23 (57.5)	(60.5)	65.22 ± 20.17
Total	**75 (100)**	**38 (50.7)**	**(100)**	**112.89 ± 22.90**
			$\chi^2 = 2.078$, ρ = 0.354	
Parity				
Nulliparity	26 (34.7)	12 (46.2)	(31.6)	225.00 ± 48.25
Primiparity	10 (13.3)	4 (40.0)	(10.5)	26.25 ± 8.26
Multiparity	39 (52.0)	22 (56.4)	(57.9)	67.50 ± 20.97
Total	**75 (100)**	**38 (50.7)**	**(100)**	**112.89 ± 22.90**
			$\chi^2 = 1.182$, ρ = 0.554	
Trimester				
2nd trimester	34 (46.6)	13 (38.2)	(36.1)	105.77 ± 39.02
3rd trimester	39 (53.4)	23 (59.0)	(63.9)	125.65 ± 30.65
Total	**73 (100)**	**36 (49.3)**	**(100)**	**118.19 ± 23.87**
			$\chi^2 = 3.126$, ρ = 0.077†	
Age Group (Year)				
< 20 ï 29	43 (57.3)	20 (46.5)	(52.6)	161.75 ± 38.62
30 ï 39	32 (42.7)	18 (56.2)	(47.4)	58.61 ± 15.12
Total	**75 (100)**	**38 (50.7)**	**(100)**	**112.89 ± 22.90**
			$\chi^2 = 0.696$, ρ = 0.404	
BMI Categories (Kg/m^2)				
18.5 ï 24.9	22 (29.3)	11 (50.0)	(28.9)	174.54 ± 6.11
25.0 ï 29.9	34 (45.3)	18 (52.9)	(47.4)	94.72 ± 23.61
Ó30	19 (25.3)	9 (47.4)	(23.7)	73.89 ± 36.19
Total	**75 (100)**	**38 (50.7)**	**(100)**	**112.89 ± 22.90**
			$\chi^2 = 0.157$, ρ = 0.925	

** Percentage for number infected for a particular maternal characteristics is represented as percentage within a subset of that category and between that subset and other subsets (i.e. within malaria).*

† indicate p value very close to 0.05. Cat.: Category.

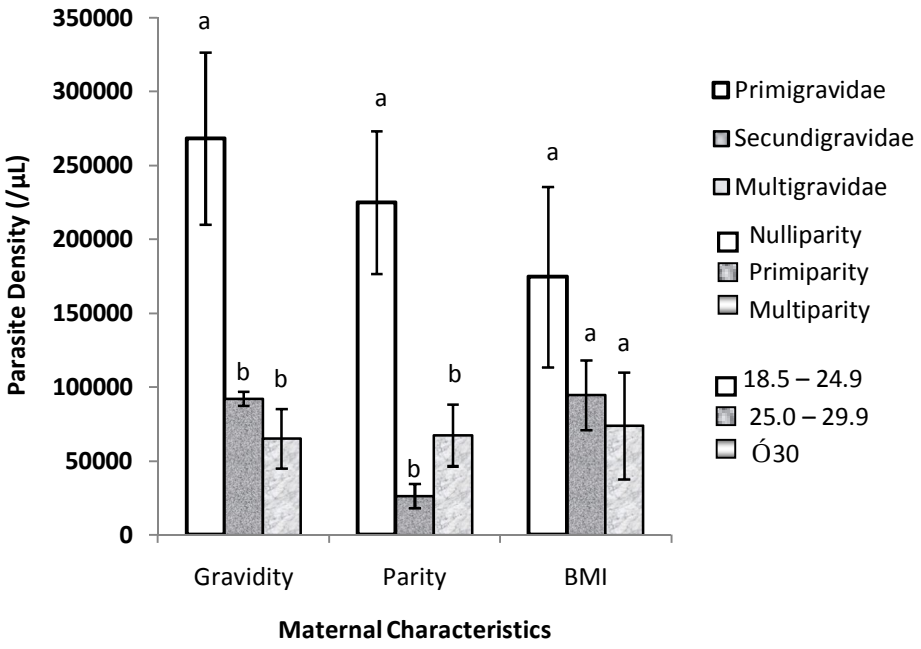

Figure 10: Comparison of parasite density by gravidity, parity and BMI

Values with different superscript across a row were significantly different (Ɉ <0.05).

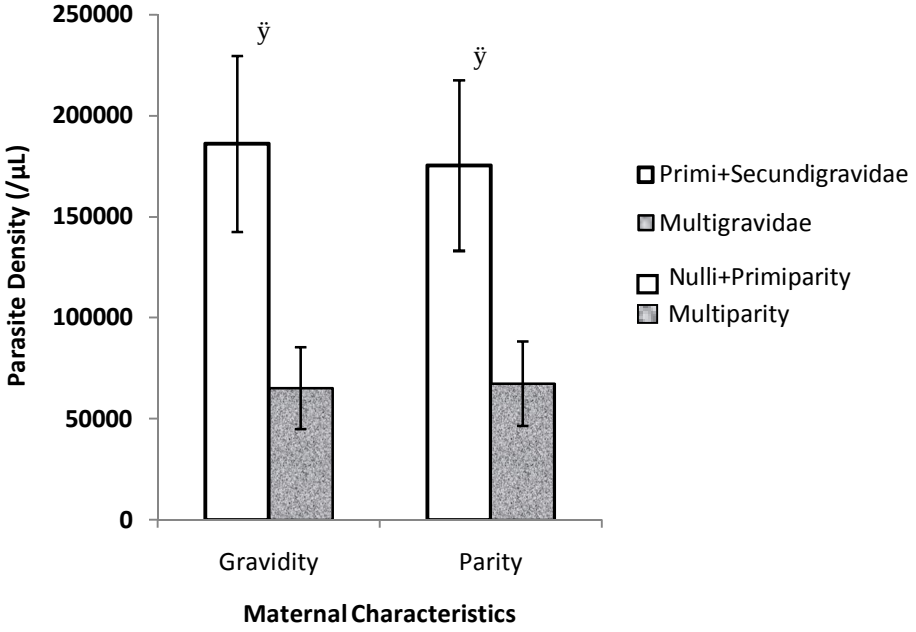

Figure 11: Comparison of parasite density by gravidity and parity

ÿ*: significantly higher at* Ɉ *< 0.05*

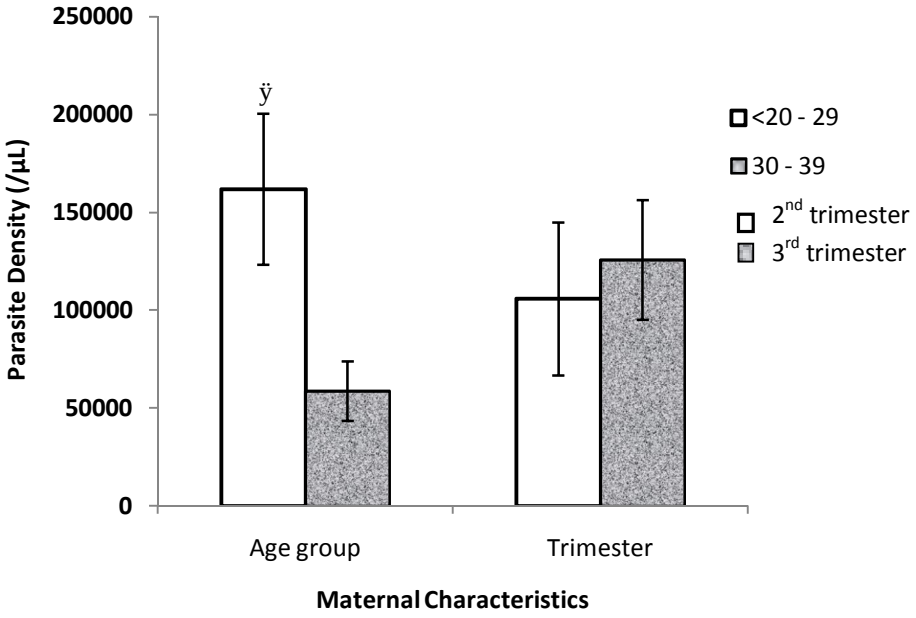

Figure 12: Comparison of parasite density by age group and trimester
ÿ*: significantly higher at* Ɉ *< 0.05*

3.2.2 Prevalence and intensity of pregnancy-associated malaria by communities

The pregnant women that participated in this study were from the following communities: Ette, Olido, Igogoro, Aji, Imufu, Umuogbu-Agu, Agu-Ibeje, Ikpamodo, Umuopu-Agu, Ufodo, Ikpiga, Ukwuinyi, Amachara, Ugbaike and Umuagama. Others include: Onicha-Enugu, Amufe, Nkpamute, Ogrute, Umuida, Umuogbo, Udah and Okata. Those communities with high antenatal attendance (Umuida, Aji and Ogrute) are represented in table 3 by pregnancy-associated malaria status.

It was observed that there were no significant differences in pregnancy-associated malaria prevalence between the women from Umuida, Ogrute and Aji ($\chi^2 = 0.108$, $p = 0.947$). There were no significant disparities at $p < 0.05$ for mean parasite density between the women from the three communities ($p = 0.890$).

When the communities were grouped as towns and villages, prevalence of pregnancy-associated malaria was slightly higher in the villages compared to towns; this disparity was not significant ($p > 0.05$). Disparity in mean parasite density was not significant between the towns and villages ($p = 0.971$).

Table 3: Prevalence and density of *falciparum* malaria parasite in pregnant women by community

	No. examined	No. Infected (%)*		Parasite
Communities	(%)	Within Comm.	Within Malaria	Density (/µL)x10^3
Umuida	10 (28.6)	6 (60.0)	(28.6)	104.17 ± 71.84
Ogrute	14 (40.0)	8 (57.1)	(38.1)	136.88 ± 50.46
Aji	11 (31.4)	7 (63.6)	(33.3)	105.71 ± 46.46
Total	**35 (100)**	**21 (60.0)**	**(100)**	**117.14 ± 30.59**
			χ^2 = 0.108, ρ = 0.947	
Communities				
Towns	34 (45.3)	17 (50.0)	(44.7)	113.82 ± 33.49
Villages	41 (54.7)	21 (51.2)	(55.3)	112.14 ± 32.08
Total	**75 (100)**	**38 (50.7)**	**(100)**	**112.89 ± 22.90**
			χ^2 = 0.011, ρ = 0.916	

** Percentage for number infected for a particular community, is represented as percentage within that community (within community) and between that community and others (i.e. within malaria).*
Comm.: Community.

3.2.3 Antenatal visits and malaria prevalence and parasite density

Thirty-seven (49.3%) of the women examined were at their first antenatal visit (Table 4). Thirty-five (95%) of these women were already past their first trimester of pregnancy at the time of first antenatal visit, while 76% were at the third trimester (Figure 13 and 14). Prevalence of pregnancy-associated malaria was 48.6% in those at their first antenatal visit and others with more than one visit. Higher malaria parasite density was observed in those with more than one antenatal visits, but the difference was not significant ($p > 0.05$).

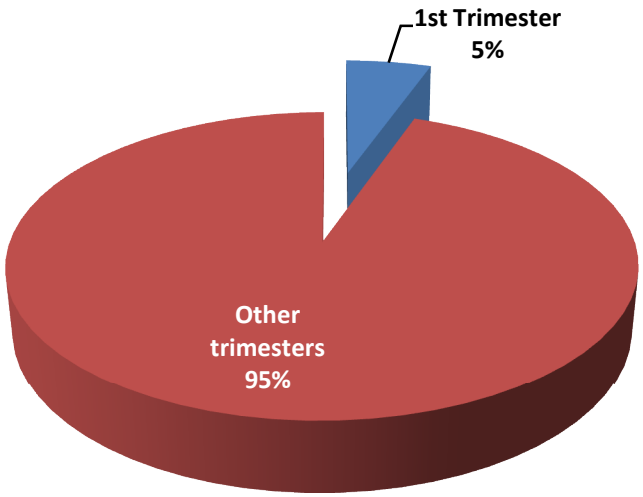

Figure 13: Trimester of pregnancy at first antenatal visit

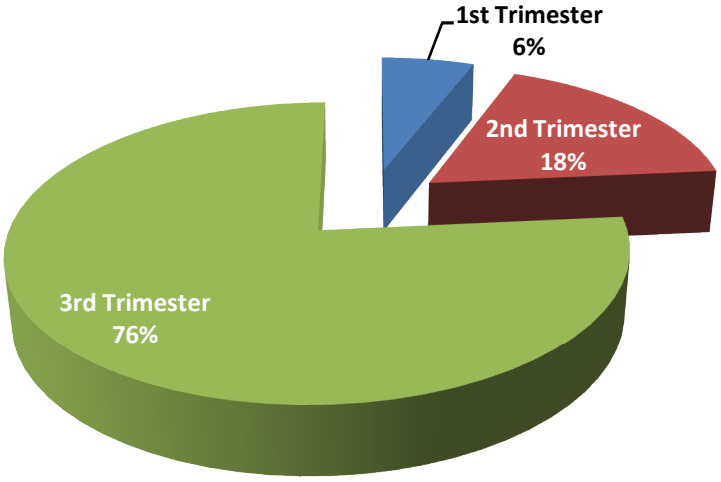

Figure 14: Trimester of pregnancy at first antenatal visit

Table 4: Prevalence and intensity of malaria by antenatal visits

Antenatal visits	No. Examined (%)	No. Infected (%)	Parasite Density (/µL)x10^3
First	37 (49.3)	18 (48.6)	75.28 ± 28.74
More than one	38 (50.7)	20 (52.6)	146.75 ± 33.86
Total	**75 (100)**	**38 (50.7)**	**112.89 ± 22.90**
		$G^2 = 0.119$, $J = 0.730$	$J = 0.121$

3.2.4 Mosquito bednet ownership and usage

Thirty-seven (49.3%) of the women examined owned mosquito bednet while only 24 (32.0%) use theirs. Ownership and usage of mosquito bednet by maternal gravidity, parity, trimester of pregnancy and age was significantly higher (ɟ < 0.05) among those at younger age (< 20, 20 - 29), primigravid and secundigravid, and nulliparous (Table 5). The multigravid women and those within age group 30 ï 39 had higher percentage bednet ownership (57.5% and 59.4% respectively) and usage (40.0% and 40.6% respectively). The duration of pregnancy also significantly determine ownership (ɟ = 0.013) but not usage (ɟ = 0.387) of mosquito bednet.

The ownership and usage of mosquito bednet in each of the community was not significantly higher than non-ownership and non-usage (ɟ > 0.05) (Table 6). The percentage ownership of bednet in town (52.9%) and usage (32.4%) was not significantly higher those in the villages 46.3% and 31.7% respectively).

Table 5: Ownership and usage of bednet by maternal clinical characteristics

	No. examined (%)	Bednet owner (%)	Bednet users (%)
Gravidity			
Primigravidae	21 (28.0)	8 (38.1)	5 (23.8)
Secundigravidae	14 (18.7)	6 (42.9)	3 (21.4)
Multigravidae	40 (53.3)	23 (57.5)	16 (40.0)
Total	**75 (100)**	**37 (49.3)**	**24 (32.0)**
		$\chi^2 = 2.363$, ρ = 0.307	$\chi^2 = 2.543$, ρ = 0.280
Parity			
Nulliparity	26 (34.7)	10 (38.5)	5 (19.2)
Primiparity	10 (13.3)	5 (50.0)	4 (40.0)
Multiparity	39 (52.0)	22 (56.4)	15 (38.5)
Total	**75 (100)**	**37 (49.3)**	**24 (32.0)**
		$\chi^2 = 2.013$, ρ = 0.366	$\chi^2 = 2.991$, ρ = 0.224
Trimester			
2nd trimester	34 (46.6)	11 (32.4)	9 (26.5)
3rd trimester	39 (53.4)	24 (61.5)	14 (35.9)
Total	**73 (100)**	**35 (47.9)**	**23 (31.5)**
		$\chi^2 = 6.199$, ρ = 0.013	$\chi^2 = 0.748$, ρ = 0.387
Age group (yr)			
< 20	9 (12.0)	1 (11.1)	1 (11.1)
20 ï 29	34 (45.3)	17 (50.0)	10 (29.4)
30 ï 39	32 (42.7)	19 (59.4)	13 (40.6)
Total	**75 (100)**	**37 (49.3)**	**24 (32.0)**
		$\chi^2 = 6.557$, ρ = 0.038	$\chi^2 = 3.003$, ρ = 0.223

Table 6: Ownership and usage of mosquito bednet by community of pregnant women

	No. examined (%)	Bednet owners (%)	Bednet users (%)
Community			
Umuida	10 (28.6)	4 (40.0)	2 (20.0)
Ogrute	14 (40.0)	7 (50.0)	4 (28.6)
Aji	11 (31.4)	4 (36.4)	3 (27.3)
Total	**35 (100)**	**15 (42.9)**	**9 (25.7)**
		$\chi^2 = 0.514$, ρ = 0.773	$\chi^2 = 0.245$, ρ = 0.885
Community			
Town	34 (45.3)	18 (52.9)	11 (32.4)
Village	41 (54.7)	19 (46.3)	13 (31.7)
Total	**75 (100)**	**37 (49.3)**	**24 (32.0)**
		$\chi^2 = 0.324$, ρ = 0.569	$\chi^2 = 0.004$, ρ = 0.952

The relationship between numbers of antenatal visits to ownership and usage of mosquito bednet, and the effect of such ownership and usage on infection by malaria parasite are indicated in figures 15 to 18. The pregnant women with more than one antenatal visit for a particular pregnancy were more likely to own and use mosquito bednet compared to those at their first antenatal attendance for a particular pregnancy, but such disparity was not significant ($p > 0.05$) (Figure 15). Presence or absence of malaria in the studied group did not appear to depend on mosquito bednet ownership and usage (Figure 16). This is as the number of malaria cases were higher than non-malaria cases among those that owned and used mosquito bednet.

The possession and use of mosquito bednet and numbers of antenatal visits were considered together as factors that may determine number of pregnancy associated malaria cases. From this it was observed that the pregnant women with more than one antenatal visits who own and use mosquito bednets also had higher cases of malaria compared to those at their first (Figure 17). But non-bednet owners at first antenatal visit had higher cases compared to those with more than one antenatal visits. The difference was not significant statistically ($p > 0.05$).

A measure of the tendency of mosquito bednet owners and users who may be infected by malaria to attend antenatal clinic is represented in figure 18. It was observed that bednet owners who had malaria infection were significantly more likely to attend antenatal ($p = 0.033$). Similarly, non-bednet owners who had malaria were significantly more likely to commence antenatal late ($p = 0.046$).

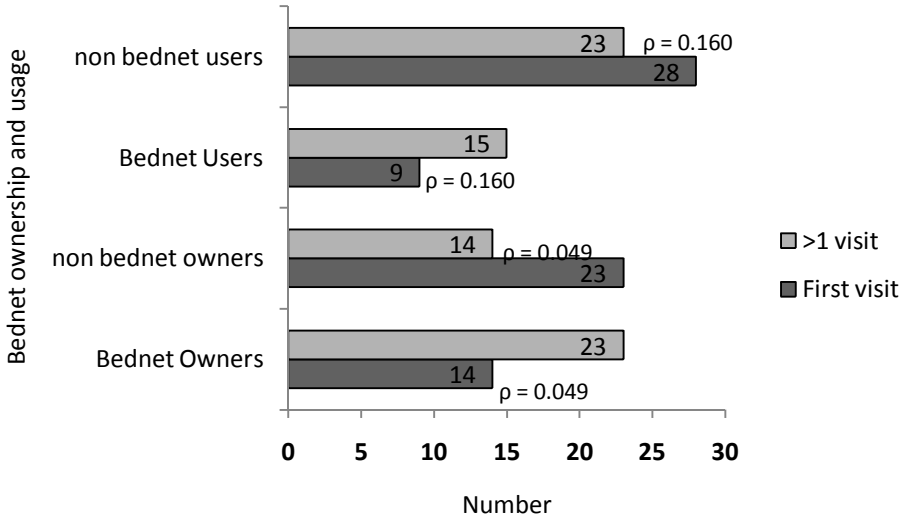

Figure 15: Bednet ownership and usage by number of antenatal visits

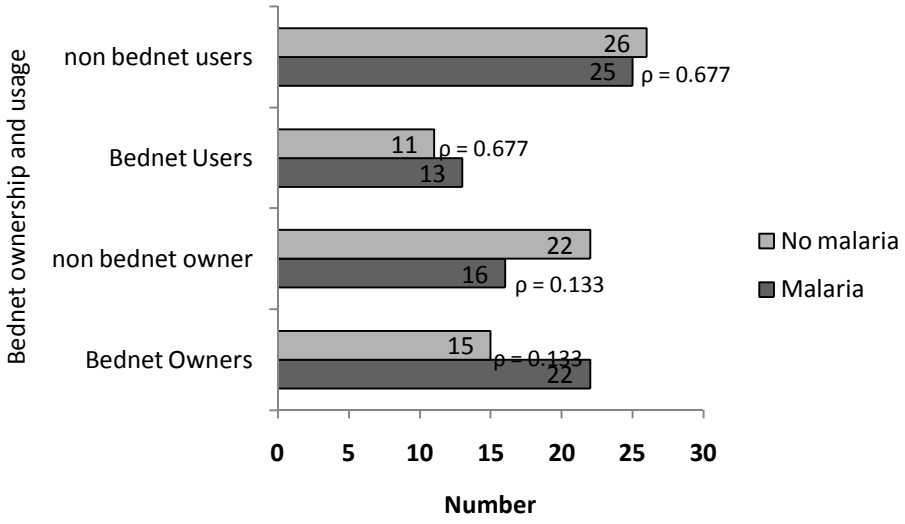

Figure 16: Mosquito bednet ownership and usage between malaria infected and uninfected women

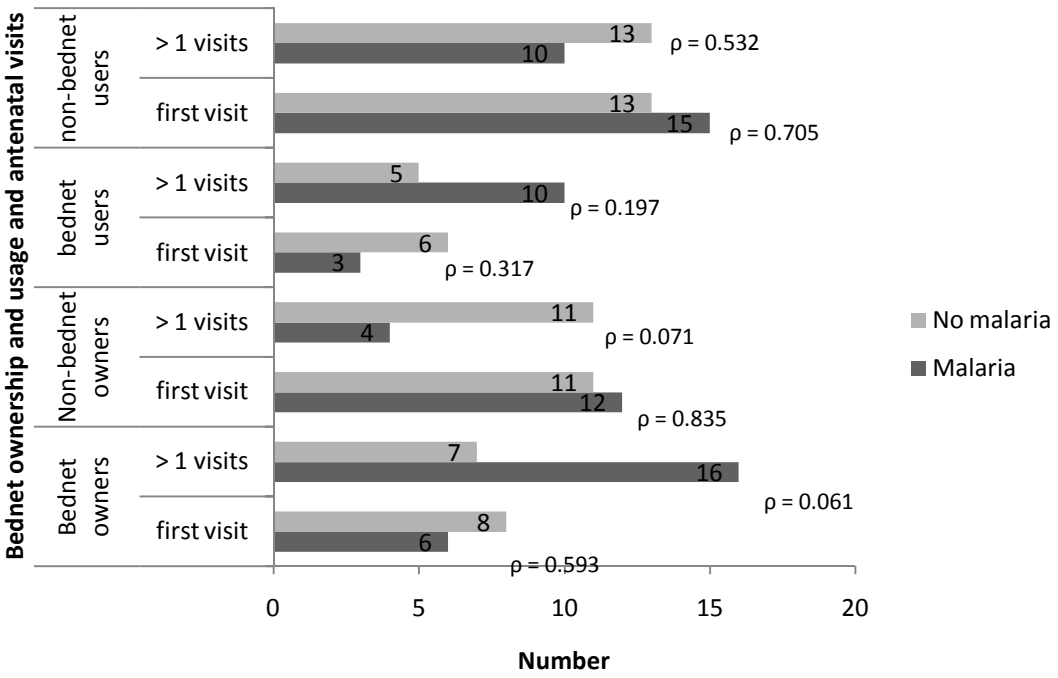

Figure 17: Number of malaria and non-malaria cases by antenatal visit and ownership and usage of mosquito bednet.

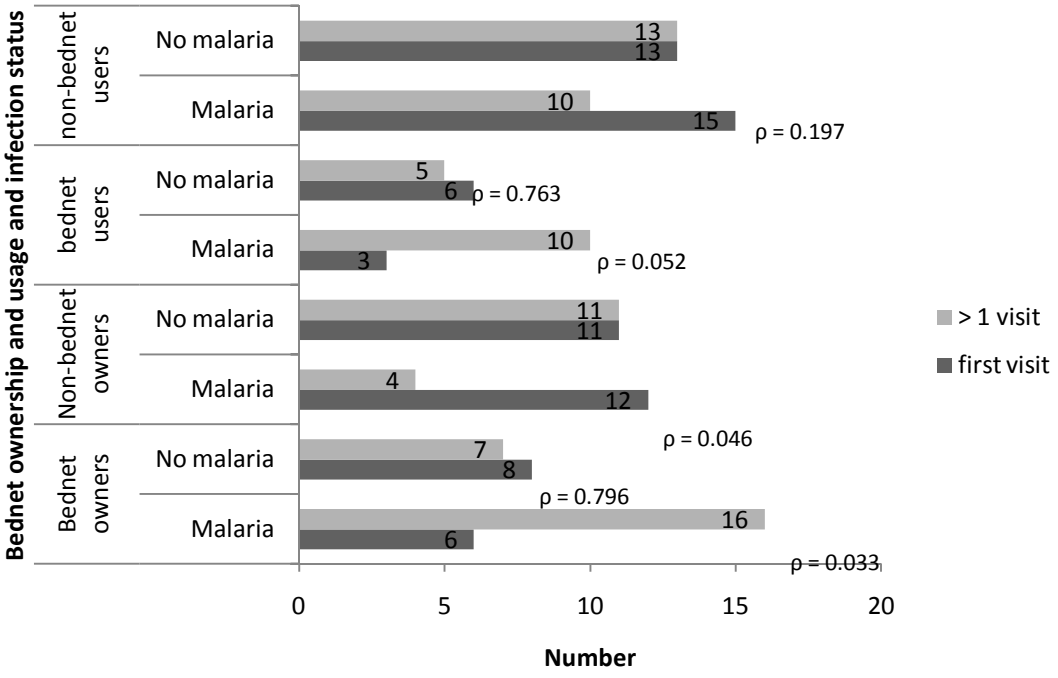

Figure 18: Antenatal visits of malaria infected and uninfected pregnant women by mosquito bednet ownership and usage.

3.2.5 Intermittent preventive malaria therapy

None of the pregnant women examined used malaria preventive therapy of any kind. The nurses also confirmed that no medication on malaria was given without confirmation of malaria case.

3.3 Maternal Obstetrics Characteristics

The result for the maternal biochemical characteristics of the pregnant women is presented here. From the Shapiro-Wilk test of normality, it was observed that the albumin to globulin ratio (A/G) was significantly skewed from normality ($p < 0.000$). As the A/G was significantly skewed from normality, Kruskal-Wallis and Mann Whitney U tests were used to determine significance difference, if any, where necessary. The results from Duncan Multiple Range tests for A/G were, however, not excluded but were taken along with others from nonparametric tests.

3.3.1 Maternal biochemical and other obstetrics characteristics by gravidity, parity and trimester of pregnancy

Comparing maternal lipid profile of primigravidae (mean age 22.95 ± 1.15 years), secundigravidae (mean age 26.92 ± 1.62 years) and multigravidae (mean age 30.38 ± 0.84years), it was observed that there were no significant difference in total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations (Table 7). LDL-C concentration of the secundigravidae was significantly higher ($p < 0.05$) than that of the primigravidae.

The total protein (TP), albumin and globulin concentrations and the albumin-globulin ratio (A/G) value were all not significantly dependent on gravidity as none was significantly different at the gravidity categories ($p > 0.05$ in all cases)(Table 7). Kruskal-Wallis test mean rank for A/G were 34.97, 37.83 and 38.54 for multigravidae, primigravidae and secundigravidae respectively and like the result from Duncan Range test, there were no significant difference ($p = 0.813$).

While no significant difference in body mass index (BMI) exist with reference to gravidity (Table 7), the BMI slightly increased from the primigravidae through the secundigravidae to the multigravidae. Gravidity was observed, however, to be very highly significantly age dependent ($p < 0.000$) with the primigravidae having the least age and the multigravidae the highest. From

comparison of age by gravidity using Bonferroni Post Hoc test, the observed difference resulted from the significant difference between the age of the primigravidae and the multigravidae ($p < 0.000$). Also, from the comparison of serum protein level using Bonferroni analysis, the observed difference resulted from the significant difference in the age of the primigravidae and secundigravidae.

The result for comparison of the biochemical characteristics of the pregnant women by parity (Table 8) closely follows the same trend observed for the comparison of the same parameters by gravidity. The mean age for the multiparous women were observed in both the sets for lipid and protein assays to be significantly higher in multiparous than nulliparous women ($p = 0.000$).

A comparison of the maternal biochemical characteristics by trimester of pregnancy, the TC, TG and LDL-C were observed to be significantly higher at the 3rd trimester of pregnancy compared to the 2nd trimester ($p = 0.047$, 0.013 and 0.036 respectively) (Table 9). The HDL-C concentration was lower at the 3rd compared to the 2nd trimester though not significantly ($p = 0.061$). The mean age and BMI of the women for lipid assay comparison by trimester of pregnancy was not significantly different ($p > 0.05$).

Total protein (TP) and globulin concentration was higher in the 3rd trimester of pregnancy compared to the second but this difference was not significant (Table 9). The lower A/G in the 3rd trimester was not significant using Mann Whitney U test ($p = 0.140$). The mean age and BMI of the women for lipid and protein assays compared by trimesters of pregnancy were not siginificantly different ($p > 0.05$)

Table 7: Comparison of maternal characteristics based on gravidity

Variables	Gravidity		
	Primigravidae	Secundigravidae	Multigravidae
Lipid	*(n =20)*	*(n =13)*	*(n =39)*
TC (mg/dl)	154.07 ± 6.22[a]	169.40 ± 8.03[a]	168.79 ± 6.46[a]
HDL-C (mg/dl)	67.02 ± 4.91[a]	59.53 ± 6.95[a]	67.61 ± 3.23[a]
TG (mg/dl)	161.12 ± 19.45[a]	154.50 ± 21.15[a]	160.92 ± 11.32[a]
LDL-C (mg/dl)	54.90 ± 6.12[b]	78.91 ± 10.56[a]	68.94 ± 6.07[ab]
Age (Year)	22.95 ± 1.15[c]	26.92 ± 1.62[b]	30.38 ± 0.84[a]
BMI (Kg/m^2)	25.95 ± 0.86[a]	27.34 ± 1.04[a]	28.37 ± 0.79[a]
Protein	*(n =21)*	*(n =14)*	*(n =37)*
TP (g/dl)	5.88 ± 0.28[a]	6.11 ± 0.39[a]	6.38 ± 0.21[a]
Albumin (g/dl)	3.21 ± 0.14[a]	3.34 ± 0.18[a]	3.41 ± 0.10[a]
Globulin (g/dl)	2.62 ± 0.29[a]	2.77 ± 0.34[a]	2.93 ± 0.20[a]
Albumin/Globulin	1.80 ± 0.32[a]	1.49 ± 0.19[a]	1.43 ± 0.14[a]
Age (Year)	22.90 ± 1.10[c]	26.57 ± 0.15[b]	30.73 ± 0.83[a]
BMI (Kg/m^2)	26.03 ± 0.82[a]	27.29 ± 0.96[a]	28.14 ± 0.73[a]

Values with different superscript across a row are significantly different ($p<0.05$).
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 8: Comparison of maternal characteristics by parity

Variables	Parity		
	Nulliparity	Primiparity	Multiparity
Lipid	*(n =25)*	*(n = 9)*	*(n = 38)*
TC (mg/dl)	156.70 ± 5.68[a]	170.47 ± 9.18[a]	169.33 ± 6.60[a]
HDL-C (mg/dl)	63.75 ± 4.73[a]	66.80 ± 7.39[a]	67.26 ± 3.40[a]
TG (mg/dl)	163.15 ± 16.97[a]	156.11 ± 26.21[a]	159.52 ± 11.20[a]
LDL-C (mg/dl)	60.38 ± 5.78[a]	72.36 ± 14.89[a]	70.10 ± 6.13[a]
Age (Year)	23.84 ± 1.08[b]	26.89 ± 2.01[ab]	30.42 ± 0.86[a]
BMI (Kg/m^2)	26.32 ± 0.80[a]	26.83 ± 1.06[a]	28.45 ± 0.81[a]
Protein	*(n =26)*	*(n =10)*	*(n =36)*
TP (g/dl)	5.84 ± 0.25[a]	6.43 ± 0.48[a]	6.36 ± 0.21[a]
Albumin (g/dl)	3.17 ± 0.13[a]	3.36 ± 0.22[a]	3.45 ± 0.09[a]
Globulin (g/dl)	2.63 ± 0.25[a]	3.03 ± 0.43[a]	2.91 ± 0.19[a]
Albumin/Globulin	1.73 ± 1.36[a]	1.41 ± 0.75[a]	1.46 ± 0.82[a]
Age (Year)	23.77 ± 1.04[b]	26.40 ± 1.86[b]	30.78 ± 0.86[a]
BMI (Kg/m^2)	26.37 ± 0.77[a]	26.82 ± 0.95[a]	28.22 ± 0.75[a]

Values with different superscript across a row are significantly different ($p<0.05$).
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 9: Comparison between maternal biochemical and clinical characteristics at different trimesters

Variables	**2nd Trimester**	**3rd Trimester**	**ρ value**
Lipid	*n = 32*	*n = 38*	
TC (mg/dl)	156.06 ± 5.94	172.63 ± 5.62	**0.047**
HDL-C (mg/dl)	71.35 ± 5.94	61.62 ± 3.44	0.061À
TG (mg/dl)	139.46 ± 13.16	183.20 ± 11.08	**0.013**
LDL-C (mg/dl)	56.66 ± 6.63	74.46 ± 5.21	**0.036**
Age (Year)	26.50 ± 1.04	28.24 ± 1.02	0.240
BMI (Kg/m^2)	28.24 ± 1.04	28.24 ± 1.02	0.789
Protein	*n = 33*	*n = 37*	
TP (g/dl)	6.07 ± 0.23	6.33 ± 0.22	0.416
Albumin (g/dl)	3.40 ± 0.12	3.26 ± 0.08	0.361
Globulin (g/dl)	2.63 ± 0.23	3.03 ± 0.19	0.183
Albumin/Globulin	1.80 ± 0.22	1.33 ± 0.12	0.140*
Age (Year)	26.18 ± 1.02	28.46 ± 1.02	0.124
BMI (Kg/m^2)	27.12 ± 0.67	27.40 ± 0.74	0.788

**significant level determined by Mann Whitney U test, others by Independent Student t test.*

n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

À*P close to 0.05*

3.3.2 Maternal biochemical and other obstetrics characteristics by age and body mass index

When the pregnant women were categorized based on age and their biochemical characteristics compared, the mean TC concentration was significantly lower in age group < 20 compared to group 20 ï 29 and group 30 ï 39 (Ɋ = 0.035). Using Bonferroni test, the observed difference resulted from TC level in the age group 30 ï 39 being higher than that in the < 20 age group by 34.08 ± 12.88 mg/dl (Ɋ = 0.030), and that of age group 20 ï 29 being higher than the < 20 age group by 27.84 ± 12.83 mg/dl (Ɋ = 0.101) (Table 10). Similarly, the BMI was still significantly less in the age group < 20. Using Bonferroni test, the statistical significant difference observed for the BMI between the age groups (Ɋ = 0.020) the result of BMI of Age group 30 ï 39 being higher than the < 20 age group by 4.69 ± 1.65 Kg/m^2 at Ɋ = 0.018.

The difference in the protein profile of the pregnant women by age was not statistically significant for the TP, albumin and globulin concentration as well as in the mean A/G. The BMI values for the age groups of the pregnant women used for protein indices determination was observed to be significant different (Ɋ = 0.007).

In table 11, a total of 22 of the pregnant women examined for lipid assays belonged to the 18.5 ï 24.5 BMI category (normal weight), 22 to the 25.0 ï 29.9 (over weight) and 19 to the Ó30 (obese), while 21, 33 and 19 women belonged to these respective categories for protein assays. There was a significant difference observed between the BMI categories for mean HDL-C (Ɋ = 0.005), LDL-C (Ɋ = 0.026) and age (Ɋ = 0.051). Using Bonferroni Post Hoc test, the statistically significant difference in HDL-C resulted solely from the HDL-C concentration of the BMI categories 18.5 ï 24.9 being less than that of the 25.0 ï 29.9 category by 18.80 ± 5.64 mg/dl (Ɋ = 0.004). For LDL-C, the BMI categories 25.0 ï 29.9 was less than the Ó30 categories by 26.93 ± 10.00 mg/dl (Ɋ = 0.027). The mean age for BMI categories 18.5 ï 24.4 was significantly less than that of the Ó30 BMI categories (Ɋ = 0.048).

Albumin/Globulin ratio was observed to be significantly different for the BMI categories (Ɋ = 0.041). Using Kruskal-Wallis Test, there was no significant difference (Ɋ = 0.131) with mean ranks of 32.29, 41.89 and 31.53 for the BMI categories 18.5 ï 24.5, 25.0 ï 29.9 and Ó 30 respectively.

Table 10: Comparison of maternal characteristics by age

	Age Group (Year)		
Variables	<20	20 ï 29	30 ï 39
Lipid	*(n = 9)*	*(n =32)*	*(n =31)*
TC (mg/dl)	138.18 ± 12.59[b]	166.02 ± 4.56[a]	172.26 ± 7.17[a]
HDL-C (mg/dl)	52.56 ± 6.20[b]	65.53 ± 4.16[ab]	70.33 ± 3.40[a]
TG (mg/dl)	190.09 ± 26.82[a]	149.97 ± 12.85[a]	161.56 ± 13.33[a]
LDL-C (mg/dl)	47.60 ± 11.06[a]	70.36 ± 5.30[a]	69.76 ± 0.76[a]
BMI (Kg/m^2)	24.03 ± 0.81[b]	27.14 ± 0.85[a]	28.72 ± 0.76[a]
Protein	*(n =9)*	*(n =32)*	*(n =31)*
TP (g/dl)	6.28 ± 0.33[a]	5.91 ± 0.25[a]	6.52 ± 0.20[a]
Albumin (g/dl)	3.28 ± 0.25[a]	3.31 ± 0.12[a]	3.46 ± 0.09[a]
Globulin (g/dl)	3.15 ± 0.39[a]	2.56 ± 0.23[a]	3.06 ± 0.20[a]
Albumin/Globulin	1.20 ± 0.19[a]	1.81 ± 0.23[a]	1.35 ± 0.14[a]
BMI (Kg/m^2)	24.03 ± 0.81[b]	26.80 ± 0.70[a]	28.72 ± 0.76[a]

Values with different superscript across a row are significantly different (p<0.05).
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 11: Comparison of maternal characteristics by BMI categories

	BMI Categories (Kg/m^2)		
Variables	18.5 ï 24.9	25.0 ï 29.9	Ó30
Lipid	*(n = 22)*	*(n =31)*	*(n =19)*
TC (mg/dl)	161.46 ± 7.20[a]	160.67 ± 5.48[a]	175.97 ± 10.16[a]
HDL-C (mg/dl)	55.76 ± 3.98[b]	74.55 ± 3.49[a]	63.81 ± 5.30[ab]
TG (mg/dl)	173.12 ± 18.14[a]	155.17 ± 12.30[a]	150.28 ± 16.98[a]
LDL-C (mg/dl)	71.08 ± 6.22[ab]	54.91 ± 5.02[b]	81.84 ± 10.83[a]
Age (Year)	25.32 ± 1.27[b]	28.00 ± 1.19[ab]	29.95 ± 1.12[a]
Protein	*(n =21)*	*(n =33)*	*(n =18)*
TP (g/dl)	6.09 ± 0.27[a]	5.97 ± 0.24[a]	6.68 ± 0.26[a]
Albumin (g/dl)	3.14 ± 0.13[a]	3.38 ± 0.11[a]	3.50 ± 0.14[a]
Globulin (g/dl)	2.95 ± 0.25[a]	2.55 ± 0.24[a]	3.18 ± 0.21[a]
Albumin/Globulin	1.34 ± 0.18[ab]	1.88 ± 0.22[a]	1.19 ± 0.09[b]
Age (Year)	25.5 ± 1.30[b]	27.64 ± 1.14[ab]	30.06 ± 1.18[a]

Values with different superscript across a row are significantly different (p<0.05).
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

3.4 Pregnancy-associated Malaria and Maternal Obstetrics Characteristics

This section covers the results of the biochemical parameters of interest for the infected and uninfected pregnant women alongside their mean age and BMI. The paremeters considered were gravidity, parity, trimesters of pregnancy, age and BMI categories.

3.4.1 Biochemical and other characteristics of malaria infected and uninfected pregnant women

The comparison of the maternal biochemical characteristics in pregnancy-associated malaria cases and the pregnant non-malaria cases is shown in table 12. Neither the plasma lipid nor the serum protein concentration was significantly different by maternal obstetrics status as it concerns the presence or absence of malaria ($p > 0.05$). The maternal lipid characteristics, though not significant at $p < 0.05$, the TC, HDL-C, TG and LDL-C concentrations were lower in malaria infected pregnant women compared to the uninfected. This observed difference was not due to the difference in age and BMI as the mean age and BMI for the infected and uninfected were almost the same.

From the set of 72 pregnant women whose protein profile were compared based on presence or absence of malaria, the total protein and globulin levels were higher in the malaria infected pregnant women. The A/G ratio of the pregnancy-associated malaria cases was lower than those without malaria, though not significantly ($p > 0.05$).

Table 12: Comparison of biochemical and other characteristics of malaria infected and uninfected women

Variables	**Malaria**		
	Present	**Absent**	**ρ value**
Lipid	*n =37*	*n =35*	
TC (mg/dl)	159.86 ± 4.58	168.62 ± 7.29	0.313
HDL-C (mg/dl)	64.12 ± 3.77	67.94 ± 3.39	0.456
TG (mg/dl)	158.82 ± 11.53	159.43 ± 13.83	0.973
LDL-C (mg/dl)	64.44 ± 4.74	69.09 ± 7.15	0.586
Age (Year)	28.24 ± 0.92	27.11 ± 1.14	0.441
BMI (Kg/m^2)	27.59 ± 0.76	27.68 ± 0.78	0.936
Protein	*n =36*	*n =36*	
TP (g/dl)	6.21 ± 0.23	6.15 ± 0.21	0.843
Albumin (g/dl)	3.28 ± 0.10	3.40 ± 0.21	0.442
Globulin (g/dl)	2.93 ± 0.21	2.72 ± 0.20	0.478
Albumin/Globulin	1.44 ± 0.15	1.66 ± 0.19	0.437*
Age (Year)	28.25 ± 0.95	27.03 ± 1.10	0.405
BMI (Kg/m^2)	27.19 ± 0.66	27.77 ± 0.74	0.562

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

3.4.2 Biochemical and other characteristics of malaria infected and uninfected pregnant women by gravidity

Tables 13, 14, 15, 16, 17 and 18 show the comparison of maternal plasma lipid, serum protein, age and BMI by gravidity between the infected and uninfected pregnant women. In table 13, no comparison of the parameters between the infected and uninfected primigravid pregnant women was significantly different ($p > 0.05$ in all the cases). The TC, HDL-C and LDL-C were unlike in table 12, higher among the malaria infected women than to the uninfected. The globulin and albumin concentration were slightly higher among the infected compared to the uninfected; but the A/G value was lower in the infected primigravidae than in their uninfected counterpart.

In the comparison of the same parameters in table 13 among the secundigravid women (Table 14) an entirely different picture in lipid concentration was obtained. There was consistency in non-significant difference ($p > 0.05$) in concentration of lipid and protein between the infected and uninfected women. TC and LDL-C concentrations were observed to be higher in the uninfected. The TG concentration and BMI value were higher in the infected than in the uninfected.

The total protein level in the infected secundigravid women was higher in the infected than in the uninfected. The globulin level remained higher just like the A/G value remained lower in the infected secundigravidae than in the uninfected.

In a comparison of the biochemical characteristics of the multigravid women (Table 15), the concentrations of TC, HDL-C, and LDL-C in the uninfected were higher than in the infected pregnant women. The TP, albumin and globulin concentrations were higher among the uninfected multigravid women, unlike the A/G, but none was significant ($p > 0.05$).

When the primigravidae and secundigravidae were combined into a new category primi+secundigravid (Table 16), the TC, TG and LDL-C levels were only slightly higher among the infected than the uninfected. The A/G value was lower in the infected women. All difference observed was significant ($p > 0.05$).

Table 13: Comparison of biochemical characteristics of malaria infected and uninfected primigravid women

Variables	Infected	Uninfected	ρ value
Lipid	*n =8*	*n =12*	
TC (mg/dl)	157.29 ± 7.02	151.92 ± 9.45	0.684
HDL-C (mg/dl)	68.17 ± 8.49	66.24 ± 9.45	0.854
TG (mg/dl)	144.67 ± 24.32	172.08 ± 28.48	0.505
LDL-C (mg/dl)	60.19 ± 1.95	51.38 ± 1.37	0.496
Age (Year)	23.50 ± 1.95	22.58 ± 1.47	0.707
BMI (Kg/m^2)	25.39 ± 0.94	26.33 ± 1.31	0.606
Protein	*n =8*	*n =13*	
TP (g/dl)	5.86 ± 0.47	5.89 ± 0.37	0.959
Albumin (g/dl)	3.22 ± 0.19	3.20 ± 0.20	0.938
Globulin (g/dl)	2.64 ± 0.40	2.61 ± 0.41	0.953
Albumin/Globulin	1.57 ± 0.39	1.93 ± 0.47	0.772*
Age (Year)	23.50 ± 1.95	22.54 ± 1.36	0.681
BMI (Kg/m^2)	25.39 ± 0.94	26.42 ± 1.21	0.553

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 14: Comparison of biochemical characteristics of malaria infected and uninfected secundigravid women

Variables	Infected	Uninfected	ρ value
Lipid	*n =7*	*n =6*	
TC (mg/dl)	166.67 ± 9.60	172.50 ± 14.18	0.734
HDL-C (mg/dl)	59.22 ± 11.48	59.88 ± 8.16	0.965
TG (mg/dl)	178.13 ± 30.16	126.93 ± 27.77	0.244
LDL-C (mg/dl)	71.90 ± 10.62	87.09 ± 19.94	0.497
Age (Year)	26.71 ± 1.49	27.17 ± 3.25	0.896
BMI (Kg/m^2)	28.63 ± 1.37	25.83 ± 1.47	0.192
Protein	*n =7*	*n =7*	
TP (g/dl)	6.57 ± 0.59	5.66 ± 0.49	0.258
Albumin (g/dl)	3.25 ± 0.29	3.44 ± 0.25	0.634
Globulin (g/dl)	3.32 ± 0.52	2.22 ± 0.35	0.105
Albumin/Globulin	1.20 ± 0.27	1.76 ± 0.25	0.085*À
Age (Year)	26.71 ± 1.49	26.43 ± 2.84	0.931
BMI (Kg/m^2)	28.63 ± 1.37	25.96 ± 1.25	0.175

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 15: Comparison of biochemical characteristics of malaria infected and uninfected multigravid women

Variables	Infected	Uninfected	ρ value
Lipid	*n =22*	*n =17*	
TC (mg/dl)	160.41 ± 6.69	180.81 ± 11.59	0.139
HDL-C (mg/dl)	64.23 ± 4.42	71.98 ± 4.65	0.239
TG (mg/dl)	159.60 ± 14.46	164.91 ± 17.97	0.817
LDL-C (mg/dl)	64.23 ± 4.42	75.84 ± 11.02	0.343
Age (Year)	30.45 ± 1.06	30.29 ± 1.39	0.926
BMI (Kg/m^2)	27.96 ± 1.45	28.89 ± 1.05	0.565
Protein	*n =21*	*n =16*	
TP (g/dl)	6.23 ± 0.30	6.58 ± 0.27	0.406
Albumin (g/dl)	3.32 ± 0.14	3.54 ± 0.13	0.257
Globulin (g/dl)	2.91 ± 0.28	3.03 ± 0.27	0.761
Albumin/Globulin	1.47 ± 0.20	1.39 ± 0.19	0.878*
Age (Year)	30.57 ± 1.11	30.94 ± 1.31	0.831
BMI (Kg/m^2)	27.30 ± 0.98	29.25 ± 1.06	0.189

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 16: Comparison of biochemical characteristics of malaria infected and uninfected primigravid+secundigravid women

Variables	Infected	Uninfected	ρ value
Lipid	*n =15*	*n =16*	
TC (mg/dl)	161.67 ± 5.78	158.78 ± 7.99	0.771
HDL-C (mg/dl)	63.99 ± 6.86	64.12 ± 4.88	0.988
TG (mg/dl)	160.28 ± 18.96	157.03 ± 21.26	0.912
LDL-C (mg/dl)	65.65 ± 6.86	63.28 ± 9.27	0.844
Age (Year)	25.00 ± 1.28	24.11 ± 1.50	0.662
BMI (Kg/m^2)	26.90 ± 0.89	26.16 ± 0.98	0.587
Protein	*n =15*	*n =18*	
TP (g/dl)	6.20 ± 0.37	5.79 ± 0.32	0.413
Albumin (g/dl)	3.23 ± 0.16	3.18 ± 0.15	0.811
Globulin (g/dl)	2.96 ± 0.32	2.55 ± 0.32	0.367
Albumin/Globulin	1.40 ± 0.24	1.82 ± 0.34	0.205*
Age (Year)	25.00 ± 1.28	24.00 ± 1.47	0.619
BMI (Kg/m^2)	26.90 ± 0.89	26.42 ± 0.96	0.718

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 17 is a comparison of the biochemical characteristics of the infected pregnant women by gravidity while table 18 is the comparison of the biochemical characteristics of the uninfected pregnant women by gravidity. In table 17, the mean age of the infected multigravidae was significantly higher than that of the primigravidae and the primigravidae+secundigravidae ($F = 5.689$, $p = 0.002$) for lipid assays group and $F = 5.677$, $p = 0.002$ for the protein assays group). Also, none of the biochemical parameters varied significantly in concentration by gravidity. The BMI of the infected by gravidity was not significantly different ($p > 0.05$).

In table 18 age was significantly different by gravidity ($F = 4.729$, $p = 0.006$ for lipid assay group) and ($F = 6.347$, $p = 0.001$ for protein assays group). TC, HDL-C, TG, LDL-C, TP, albumin, globulin, A/G and BMI were not significantly different by gravidity ($p > 0.05$) though there were variations in the levels of these parameters by gravidity.

Table 17: Comparison of biochemical characteristics of infected pregnant women by gravidity

	Gravidity (Infected)			
Variables	Primigravidae	Secundigravidae	Multigravidae	Primi+Secundigravidae*
Lipid	*n =8*	*n =7*	*n =22*	*n =15*
TC (mg/dl)	157.29 ± 7.02[a]	166.67 ± 9.66[a]	160.41 ± 6.69[a]	161.67 ± 5.78[a]
HDL-C (mg/dl)	68.17 ± 8.49[a]	59.22 ± 11.48[a]	64.23 ± 4.42[a]	63.99 ± 3.29[a]
TG (mg/dl)	144.67 ± 24.32[a]	178.13 ± 30.16[a]	159.60 ± 14.46[a]	160.28 ± 18.96[a]
LDL-C (mg/dl)	60.19 ± 9.09[a]	71.90 ± 10.62[a]	64.17 ± 6.49[a]	65.65 ± 6.86[a]
Age (Year)	23.50 ± 1.95[b]	26.71 ± 1.49[ab]	30.45 ± 1.06[a]	25.00 ± 1.28[b]
BMI (Kg/m^2)	25.39 ± 0.94[a]	28.63 ± 1.37[a]	27.96 ± 1.15[a]	26.90 ± 0.89[a]
Protein	*n =8*	*n =7*	*n =21*	*n =15*
TP (g/dl)	5.86 ± 0.48[a]	6.57 ± 0.59[a]	6.23 ± 0.30[a]	6.20 ± 0.37[a]
Albumin (g/dl)	3.22 ± 0.19[a]	3.25 ± 0.29[a]	3.32 ± 0.14[a]	3.23 ± 0.16[a]
Globulin (g/dl)	2.64 ± 0.40[a]	3.32 ± 0.52[a]	2.91 ± 0.28[a]	2.96 ± 0.32[a]
Albumin/Globulin	1.57 ± 0.39[a]	1.20 ± 0.27[a]	1.47 ± 0.20[a]	1.40 ± 0.93[a]
Age (Year)	23.50 ± 1.95[b]	26.71 ± 1.49[ab]	30.57 ± 1.11[a]	25.00 ± 1.28[b]
BMI (Kg/m^2)	25.39 ± 0.94[a]	28.63 ± 1.37[a]	27.30 ± 0.98[a]	26.90 ± 0.89[a]

Values with different superscript across a row are significantly different ($p<0.05$).
*n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index. * primi+secundigravidae was obtained by the combination of the primigravidae and secundigravidae.*

Table 18: Comparison of biochemical characteristics of uninfected pregnant women by gravidity

	Gravidity (Uninfected)			
Variables	Primigravidae	Secundigravidae	Multigravidae	Primi+Secundigravidae*
Lipid	*n =12*	*n =6*	*n =17*	*n =18*
TC (mg/dl)	151.92 ± 9.45[a]	172.50 ± 14.18[a]	180.81 ± 11.59[a]	158.78 ± 7.99[a]
HDL-C (mg/dl)	66.24 ± 6.23[a]	59.88 ± 8.16[a]	71.98 ± 4.65[a]	64.12 ± 4.88[a]
TG (mg/dl)	172.08 ± 28.48[a]	126.93 ± 27.77[a]	164.91 ± 17.97[a]	157.03 ± 21.26[a]
LDL-C (mg/dl)	51.38 ± 8.37[a]	87.09 ± 19.94[a]	75.84 ± 11.02[a]	63.28 ± 9.27[a]
Age (Year)	22.58 ± 1.47[b]	27.17 ± 3.25[ab]	30.29 ± 1.39[a]	24.11 ± 1.50[b]
BMI (Kg/m^2)	26.33 ± 1.31[a]	25.83 ± 1.47[a]	28.89 ± 1.05[a]	26.16 ± 0.98[a]
Protein	*n =13*	*n =7*	*n =16*	*n =20*
TP (g/dl)	5.89 ± 0.37[a]	5.66 ± 0.49[a]	6.58 ± 0.27[a]	5.81 ± 0.29[a]
Albumin (g/dl)	3.20 ± 0.20[a]	3.44 ± 0.25[a]	3.54 ± 0.13[a]	3.28 ± 0.15[a]
Globulin (g/dl)	2.61 ± 0.41[a]	2.22 ± 0.35[a]	3.54 ± 0.13[a]	3.28 ± 0.15[a]
Albumin/Globulin	1.93 ± 0.47[a]	1.76 ± 0.25[a]	1.39 ± 0.19[a]	1.87 ± 0.31[a]
Age (Year)	22.54 ± 1.36[b]	26.43 ± 2.84[ab]	30.94 ± 1.31[a]	23.90 ± 1.35[b]
BMI (Kg/m^2)	26.42 ± 1.21[a]	25.96 ± 1.25[a]	29.25 ± 1.06[a]	26.26 ± 1.35[a]

Values with different superscript across a row are significantly different ($p<0.05$).
*n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index. * primi+secundigravidae was obtained by the combination of the primigravidae and secundigravidae.*

3.4.3 Biochemical and other characteristics of malaria infected and uninfected pregnant women by Parity

The maternal obstetrics characteristics of interest between malaria infected and uninfected women were compared by parity in tables 19 - 23. In table 19, the mean TC level was within the same range in the infected and uninfected nulliparous women (ɟ > 0.05). The HDL-C and TG in the uninfected nulliparous pregnant women were not significantly higher than the HDL-C and TG in the infected women. Mean age and BMI between the infected and uninfected nulliparous women were not significantly different (ɟ > 0.05). The A/G value of the infected nulliparous women was less than that of their uninfected counterpart, but the difference was not significant from Mann Whitney U test (ɟ > 0.05).

Among the multiparous infected and uninfected women (Table 20), the mean TC, HDL-C, TG and LDL-C concentration were less in the malaria infected group than in their uninfected counterpart. The mean age and BMI of the multiparous women whose lipid profile were compared did not differ significantly between the infected and uninfected (ɟ > 0.05). The mean TP and globulin concentrations in the infected multiparous women were less than in the uninfected, but the difference was not significant in both cases (ɟ > 0.05). The Albumin-globulin ratio (A/G) was slightly higher in the infected multiparous women than their uninfected counterpart (P > 0.05).

In table 21, the obstetrics characteristics in infected and uninfected pregnant nulliparous and primiparous women combined (nulli+primiparous) were compared. There were no significant differences observed for any parameter between the infected and uninfected women (ɟ > 0.05). The A/G in the infected women remained lower than in the unifected. This was not significant (ɟ > 0.05).

Table 19: Comparison of biochemical characteristics of malaria infected and uninfected nulliparous women

Variables	Infected	Uninfected	ρ value
Lipid	*n =12*	*n =13*	
TC (mg/dl)	159.39 ± 7.05	154.22 ± 9.00	0.658
HDL-C (mg/dl)	62.75 ± 7.75	64.64 ± 5.96	0.847
TG (mg/dl)	147.84 ± 20.62	177.28 ± 26.71	0.398
LDL-C (mg/dl)	67.09 ± 8.04	54.18 ± 8.20	0.274
Age (Year)	24.17 ± 1.44	23.54 ± 1.66	0.779
BMI (Kg/m^2)	26.67 ± 1.02	26.01 ± 1.25	0.689
Protein	*n =12*	*n =14*	
TP (g/dl)	5.81 ± 0.37	5.87 ± 0.34	0.905
Albumin (g/dl)	3.13 ± 0.18	3.21 ± 0.18	0.758
Globulin (g/dl)	2.69 ± 0.35	2.66 ± 0.38	0.961
Albumin/Globulin	1.53 ± 0.29	1.83 ± 0.43	0.887*
Age (Year)	24.17 ± 1.44	23.43 ± 1.54	0.732
BMI (Kg/m^2)	26.67 ± 1.02	26.12 ± 1.16	0.731

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 20: Comparison of biochemical characteristics of malaria infected and uninfected multiparous women

Variables	Infected	Uninfected	ρ value
Lipid	*n =12*	*n =17*	
TC (mg/dl)	161.79 ± 6.91	180.83 ± 11.53	0.170
HDL-C (mg/dl)	63.88 ± 4.67	71.98 ± 4.65	0.216
TG (mg/dl)	154.84 ± 13.77	164.91 ± 19.97	0.653
LDL-C (mg/dl)	66.84 ± 6.50	75.84 ± 11.02	0.464
Age (Year)	30.00 ± 1.18	30.29 ± 1.39	0.872
BMI (Kg/m^2)	27.77 ± 1.18	28.89 ± 1.05	0.497
Protein	*n =20*	*n =16*	
TP (g/dl)	6.19 ± 0.31	6.48 ± 0.31	0.516
Albumin (g/dl)	3.40 ± 0.14	3.49 ± 0.15	0.647
Globulin (g/dl)	2.81 ± 0.28	2.93 ± 0.29	0.780
Albumin/Globulin	1.52 ± 0.20	1.40 ± 0.19	0.691*
Age (Year)	30.65 ± 1.16	30.94 ± 1.31	0.870
BMI (Kg/m^2)	27.39 ± 1.03	29.25 ± 1.06	0.220

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 21: Comparison of biochemical characteristics of malaria infected and uninfected women of nulliparous+primiparous category

Variables	**Infected**	**Uninfected**	**ρ value**
Lipid	*n =16*	*n =18*	
TC (mg/dl)	162.11 ± 5.42	152.78 ± 7.99	0.732
HDL-C (mg/dl)	65.06 ± 6.50	64.09 ± 4.89	0.905
TG (mg/dl)	166.07 ± 18.66	157.03 ± 21.26	0.754
LDL-C (mg/dl)	63.85 ± 6.67	63.28 ± 9.27	0.961
Age (Year)	25.25 ± 1.22	24.11 ± 1.50	0.566
BMI (Kg/m^2)	26.81 ± 0.84	26.16 ± 0.98	0.621
Protein	*n =16*	*n =20*	
TP (g/dl)	6.25 ± 0.35	5.81 ± 0.29	0.343
Albumin (g/dl)	3.18 ± 0.16	3.28 ± 0.15	0.651
Globulin (g/dl)	3.07 ± 0.32	2.53 ± 0.29	0.222
Albumin/Globulin	1.35 ± 0.23	1.82 ± 0.31	0.139*
Age (Year)	25.25 ± 1.22	23.90 ± 1.35	0.475
BMI (Kg/m^2)	26.81 ± 0.84	26.26 ± 0.88	0.658

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 22 and 23 were the results when malaria infection in the infected and uninfected women was controlled for while comparing the characteristics of interest by parity. While comparing the biochemical attributes of the infected women by parity taking note of their mean age and BMI (Table 22), it was observed that TG concentration in the nulliparous women was significantly less than that in the primiparous women. The mean age of the multiparous pregnanant women whose lipid profile were compared was significantly higher than what was obtained in the nulliparous and nulli+primiparous ($p = 0.002$).

From the section of table 22 where protein profile of the women were compared, the concentration of TP and globulin were significantly higher ($p < 0.05$) in the primiparous than the nulliparous and multiparous, but this significance was eliminated when the nulliparous and primiparous women were combined (nulli+primiparous). The mean age of this group remained higher in the multiparous than the nulliparous and the nulli+primiparous ($p < 0.05$).

In table 23, where the same parameters as table 22 were compared among the uninfected by parity, only the age was observed to retain significant variation ($p < 0.05$).

Table 22: Comparison of biochemical and other characteristics of infected pregnant women by parity

	Parity (Infected)			
Variables	Nulliparity	Primiparity	Multiparity	Nulli+Primiparity*
Lipid	*n =12*	*n =4*	*n =21*	*n =16*
TC (mg/dl)	159.39 ± 7.05^{a}	170.23 ± 3.42^{a}	160.04 ± 7.01^{a}	162.11 ± 5.42^{a}
HDL-C (mg/dl)	62.75 ± 7.75^{a}	71.98 ± 12.80^{a}	63.43 ± 4.56^{a}	65.06 ± 6.50^{a}
TG (mg/dl)	147.84 ± 20.62^{b}	220.75 ± 30.96^{a}	155.16 ± 14.43^{ab}	166.07 ± 18.66^{ab}
LDL-C (mg/dl)	67.09 ± 8.04^{a}	54.15 ± 1.56^{a}	65.47 ± 6.67^{a}	63.85 ± 6.67a
Age (Year)	24.17 ± 1.44^{b}	28.50 ± 1.56^{ab}	30.52 ± 1.11^{a}	25.25 ± 1.22^{b}
BMI (Kg/m^{2})	26.67 ± 1.02^{a}	27.25 ± 1.60^{a}	28.08 ± 1.20^{a}	26.81 ± 0.84^{a}
Protein	*n =12*	*n =4*	*n =20*	*n =16*
TP (g/dl)	5.81 ± 0.37^{b}	7.56 ± 0.90^{a}	6.19 ± 0.31^{b}	6.25 ± 0.35^{b}
Albumin (g/dl)	3.13 ± 0.18^{a}	3.33 ± 0.37^{a}	3.40 ± 0.14^{a}	3.18 ± 0.16^{a}
Globulin (g/dl)	2.69 ± 0.35^{b}	4.23 ± 0.38^{a}	3.07 ± 0.28^{b}	3.07 ± 0.32^{b}
Albumin/Globulin*	1.52 ± 0.29^{a}	0.83 ± 0.14^{a}	1.52 ± 0.20^{a}	1.35 ± 0.23^{a}
Age (Year)	24.17 ± 1.44^{b}	28.50 ± 1.56^{ab}	30.65 ± 1.16^{a}	25.25 ± 1.22^{b}
BMI (Kg/m^{2})	26.67 ± 1.02^{a}	27.25 ± 1.60^{a}	27.39 ± 1.03^{a}	26.81 ± 0.84^{a}

Values with different superscript across a row are significantly different ($p<0.05$).
*n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index. * nulli+primiparity was obtained by the combination of the nulliparity and primiparity.*

Table 23: Comparison of biochemical and other characteristics of uninfected pregnant women by parity

	Parity (Uninfected)			
Variables	Nulliparity	Primiparity	Multiparity	Nulli+Primiparity*
Lipid	*n =13*	*n =5*	*n =17*	*n =18*
TC (mg/dl)	154.22 ± 9.00^{a}	170.63 ± 17.21^{a}	180.83 ± 11.58^{a}	158.78 ± 7.99^{a}
HDL-C (mg/dl)	64.64 ± 5.96^{a}	62.66 ± 9.40^{a}	71.98 ± 4.65^{a}	64.09 ± 4.89^{a}
TG (mg/dl)	177.28 ± 26.71^{a}	104.39 ± 19.86^{a}	164.91 ± 17.97^{a}	157.03 ± 21.26^{a}
LDL-C (mg/dl)	54.18 ± 8.20^{a}	86.93 ± 24.42^{a}	75.84 ± 11.02^{a}	63.28 ± 9.27^{a}
Age (Year)	23.54 ± 1.66^{b}	25.60 ± 3.49^{ab}	30.29 ± 1.39^{a}	24.11 ± 1.50^{b}
BMI (Kg/m^{2})	26.01 ± 1.25^{a}	26.56 ± 1.57^{a}	28.89 ± 1.05^{a}	26.16 ± 0.98^{a}
Protein	*n =14*	*n =6*	*n =16*	*n =20*
TP (g/dl)	5.87 ± 0.34^{a}	5.68 ± 0.58^{a}	6.48 ± 0.31^{a}	5.81 ± 0.29^{a}
Albumin (g/dl)	3.21 ± 0.18^{a}	3.44 ± 0.30^{a}	3.49 ± 0.15^{a}	3.28 ± 0.15^{a}
Globulin (g/dl)	2.66 ± 0.38^{a}	2.23 ± 0.41^{a}	2.93 ± 0.29^{a}	2.53 ± 0.29^{a}
Albumin/Globulin*	1.83 ± 0.43^{a}	1.79 ± 0.29^{a}	1.40 ± 0.19^{a}	1.82 ± 0.31^{a}
Age (Year)	23.43 ± 1.54^{b}	25.00 ± 2.91^{b}	30.94 ± 1.31^{a}	23.90 ± 1.35^{b}
BMI (Kg/m^{2})	26.12 ± 1.16^{a}	26.58 ± 1.28^{a}	29.25 ± 1.06^{a}	26.26 ± 0.88^{a}

Values with different superscript across a row are significantly different ($p<0.05$).
*n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index. * nulli+primiparity was obtained by the combination of the nulliparity and primiparity.*

3.4.4 Biochemical and other characteristics of malaria infected and uninfected pregnant women by trimester of pregnancy

The obstetrics characteristics of interest among the infected and uninfected women by trimesters of pregnancy were compared in table 24. In the aspect of table 24 covering the second trimester, the mean concentration of TC, HDL-C, TG and LDL-C just like age and BMI were higher in the infected than in the uninfected women at the second trimester of pregnancy, but the difference were not significant ($p > 0.05$). The mean total protein, albumin and globulin concentration as well as mean age and BMI were higher in the infected women than the uninfected at the second trimester ($p > 0.05$ in all cases). The A/G value was, however, less in the infected but not significantly ($p > 0.05$).

At the 3rd trimester of pregnancy, it was observed that TC concentration in malaria infected women was significantly less than that of their uninfected counterpart ($p = 0.004$). The LDL-C concentration was also significantly lower in the malaria infected women ($p = 0.025$). The albumin concentration was also appreciably low in the infected women than the unifected even though not significant at $p < 0.05$ ($p = 0.099$).

Table 24: Comparison of biochemical characteristics of malaria infected and uninfected women by trimester of pregnancy

Trimesters							
2nd Trimester				3rd Trimester			
Variables	**Infected**	**uninfected**	**ρ value**	**Variables**	**Infected**	**Uninfected**	**ρ value**
Lipid	*n = 13*	*n = 19*			*n = 22*	*n = 16*	
TC (mg/dl)	164.70 ± 8.39	150.14 ± 8.10	0.235	TC (mg/dl)	158.26 ± 4.95	192.43 ± 9.65	**0.004**
HDL-C (mg/dl)	74.33 ± 5.04	69.81 ± 5.40	0.565	HDL-C (mg/dl)	58.23 ± 5.17	66.28 ± 3.91	0.253
TG (mg/dl)	134.35 ± 19.28	142.22 ± 18.09	0.773	TG (mg/dl)	184.23 ± 12.51	182.11 ± 20.42	0.927
LDL-C (mg/dl)	63.19 ± 9.86	52.21 ± 8.97	0.425	LDL-C (mg/dl)	63.32 ± 4.82	89.01 ± 9.55	**0.025**
Age (Year)	28.00 ± 1.24	25.68 ± 1.55	0.251	Age (Year)	27.82 ± 1.32	28.81 ± 1.64	0.637
BMI (Kg/m^2)	28.65 ± 1.74	27.21 ± 0.75	0.460	BMI (Kg/m^2)	26.72 ± 0.80	27.83 ± 1.39	0.495
Protein	*n = 12*	*n = 21*			*n = 22*	*n = 15*	
TP (g/dl)	6.21 ± 0.35	5.99 ± 0.30	0.653	TP (g/dl)	6.29 ± 0.32	6.38 ± 0.28	0.852
Albumin (g/dl)	3.49 ± 0.21	3.35 ± 0.16	0.586	Albumin (g/dl)	3.18 ± 0.11	3.47 ± 0.12	0.099À
Globulin (g/dl)	2.71 ± 0.37	2.59 ± 0.30	0.794	Globulin (g/dl)	3.11 ± 0.27	2.91 ± 0.26	0.606
Albumin/Globulin	1.69 ± 0.30	1.87 ± 0.31	0.911*	Albumin/Globulin	1.31 ± 0.18	1.36 ± 0.15	0.448*
Age (Year)	28.00 ± 1.34	25.33 ± 1.42	0.221	Age (Year)	27.82 ± 1.32	29.40 ± 1.63	0.455
BMI (Kg/m^2)	27.54 ± 1.47	27.21 ± 0.68	0.837	BMI (Kg/m^2)	26.72 ± 0.80	28.14 ± 1.45	0.401

**significant level determined by Mann Whitney U test, others by Independent Student t test.*

n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

À*P close to 0.05*

3.4.5 Biochemical and other characteristics of malaria infected and uninfected pregnant women by age

From table 25, a comparison of the aforementioned parameters between the infected and uninfected pregnant women within the age group 20 ï 29, the TC, HDL-C and LDL-C concentration in the uninfected women were higher than in the infected, but the variations were not significant at Ɉ < 0.05. Triglyceride level was higher in the malaria infected of this age group category. The total protein level was higher in the infected group compared to the uninfected in the 20 ï 29 age group (Ɉ = 0.171). The globulin level was appreciably higher in the infected women of this age group compared to the uninfected even though Ɉ > 0.05 (Ɉ = 0.067).

The observation for the age group 30 ï 39 was similar in many ways to that in the age group 20 ï 29. The TC, HDL-C and LDL-C concentration were lower in the infected compared to the uninfected though none was significant at Ɉ < 0.05. The mean BMI of the infected was significantly less than that for the uninfected (Ɉ = 0.047). The TP concentration in the infected women in this age group was significantly less than the TP in the uninfected (P = 0.047). The albumin level in the infected women was much less than that of the uninfected even though it was not significant at Ɉ < 0.05. The mean BMI of the infected women was also significantly less than that of the uninfected (Ɉ = 0.047).

In order to ascertain whether malaria infection can significantly alter the biochemical and BMI characteristics of malaria infected pregnant women than age disparity can, malaria infection was controlled for in table 26. The TC, HDL-C and TG concentrations for the age group 30 ï 39 were higher than in group 20 ï 29, but no significant difference was observed (Ɉ > 0.05). No significant variation between the TP, albumin, globulin, A/G and BMI values for the 20 -29 and 30 -39 or <20 -29 and the 30 -39 age group was observed. The parameters retained similar comparative values as in table 10, though there slight variations.

Table 25: Comparison of biochemical characteristics of malaria infected and uninfected women by age at pregnancy

Age Group (Year)							
< 20 ï 29				30 ï 39			
Variables	**Infected**	**Uninfected**	**ρ value**	**Variables**	**Infected**	**Uninfected**	**ρ value**
Lipid	*n = 18*	*n = 14*			*n = 22*	*n = 16*	
TC (mg/dl)	158.90 ± 5.66	173.04 ± 7.94	0.147	TC (mg/dl)	165.78 ± 7.63	180.12 ± 12.91	0.350
HDL-C (mg/dl)	60.90 ± 5.30	71.48 ± 6.50	0.212	HDL-C (mg/dl)	69.62 ± 5.65	71.20 ± 3.36	0.813
TG (mg/dl)	151.53 ± 15.21	147.97 ± 22.58	0.893	TG (mg/dl)	166.49 ± 18.50	153.17 ± 19.81	0.628
LDL-C (mg/dl)	67.47 ± 6.71	71.96 ± 9.35	0.691	LDL-C (mg/dl)	62.98 ± 7.47	78.28 ± 13.21	0.324
Age (Year)	24.50 ± 0.67	23.79 ± 0.77	0.490	Age (Year)	33.35 ± 0.62	34.50 ± 0.56	0.191
BMI (Kg/m^2)	27.96 ± 1.38	26.09 ± 0.78	0.285	BMI (Kg/m^2)	27.36 ± 0.83	30.38 ± 1.25	**0.047**
Protein	*n = 17*	*n = 14*			*n = 17*	*n = 14*	
TP (g/dl)	6.24 ± 0.34	5.54 ± 0.36	0.171	TP (g/dl)	6.06 ± 0.34	6.89 ± 0.21	**0.049**
Albumin (g/dl)	3.27 ± 0.17	3.35 ± 0.16	0.713	Albumin (g/dl)	3.26 ± 0.14	3.62 ± 0.12	0.068À
Globulin (g/dl)	2.97 ± 0.30	2.11 ± 0.32	0.057À	Globulin (g/dl)	2.80 ± 0.33	3.27 ± 0.23	0.254
Albumin/Globulin	1.40 ± 0.22	2.27 ± 0.39	0.067*À	Albumin/Globulin	1.54 ± 0.24	1.21 ± 0.56	0.463*
Age (Year)	24.27 ± 0.68	23.80 ± 0.69	0.615	Age (Year)	33.35 ± 0.62	34.50 ± 0.56	0.191
BMI (Kg/m^2)	27.14 ± 1.18	26.43 ± 0.70	0.609	BMI (Kg/m^2)	27.36 ± 0.83	30.38 ± 1.25	**0.047**

**significant level determined by Mann Whitney U test, others by Independent Student t test.*

n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

À*P close to 0.05*

Table 26: Comparison of biochemical characteristics of infected women by age groups

Variables	**Age Group (Year)**		
	20 -29	*30 – 39*	*<20 – 29*
Lipid	*(n =18)*	*(n =16)*	*(n =20)*
TC (mg/dl)	158.90 ± 5.66[a]	163.75 ± 7.83[a]	156.82 ± 5.42[a]
HDL-C (mg/dl)	60.90 ± 5.29[a]	69.45 ± 6.01[a]	59.47 ± 3.10[a]
TG (mg/dl)	151.53 ± 15.21[a]	166.69 ± 19.69[a]	154.26 ± 14.29[a]
LDL-C (mg/dl)	67.47 ± 6.71[a]	61.09 ± 7.69[a]	66.29 ± 6.09[a]
BMI (Kg/m^2)	27.96 ± 1.38[a]	27.26 ± 0.88[a]	27.68 ± 1.25[a]
Protein	*(n =17)*	*(n =16)*	*(n =19)*
TP (g/dl)	6.24 ± 0.34[a]	6.16 ± 0.35[a]	6.35 ± 0.32[a]
Albumin (g/dl)	3.27 ± 0.17[a]	3.26 ± 0.15[a]	3.31 ± 0.15[a]
Globulin (g/dl)	2.97 ± 0.30[a]	2.90 ± 0.33[a]	3.04 ± 0.27[a]
Albumin/Globulin	1.40 ± 0.22[a]	1.48 ± 0.24[a]	1.36 ± 0.20[a]
BMI (Kg/m^2)	27.14 ± 1.18[a]	27.26 ± 0.88[a]	26.93 ± 1.06[a]

Values with different superscript across a row are significantly different ($p<0.05$).
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

3.4.6 Biochemical and other characteristics of malaria infected and uninfected pregnant women by BMI

Table 27, 28 and 29 show the results for the comparison of the biochemical characteristics of the pregnant women by BMI categories. In table 27, there were no significant difference (Ɉ > 0.05) observed for all the parameters between the infected and uninfected for the BMI category 18.5 ï 24.9. In this BMI category, the TG level in the uninfected was higher than that in the infected women. The A/G value was lower in the infected than in the uninfected women, though not significantly (Ɉ > 0.05).

In the BMI category 25.0 ï 29.9 (Table 28), the mean age of the malaria infected pregnant women was significantly higher than that of the uninfected (Ɉ < 0.05) for both the group whose plasma lipid and those whose serum protein were assayed. The TC, TG, and LDL-C concentrations were observed to be higher in the malaria infected women than their uninfected counterpart, but in all these cases, the difference was not significant (Ɉ > 0.05). The mean albumin, globulin and A/G values were less in the infected pregnant women.

The TC concentration of the malaria infected pregnant women was significantly lower than the uninfected in the BMI category Ó30 (Ɉ = 0.023) (Table 29). The HDL-C concentration was also much lower in the infected, with Ɉ very close to 0.05 (Ɉ = 0.058). The ages of the infected and uninfected were significantly different (Ɉ = 0.008) with the uninfected women having a higher mean age. The Total protein, albumin and globulin concentrations were less in the infected women, but the A/G value was less in the uninfected. Apart from the age in the groups compared for protein parameters, no other parameter was significantly different between the infected and uninfected.

Table 27: Comparison of biochemical characteristics of malaria infected and uninfected women within BMI 18.5 ï 24.9

Variables	**Infected**	**Uninfected**	**ρ value**
Lipid	*n =11*	*n =11*	
TC (mg/dl)	160.56 ± 9.68	162.86 ± 10.75	0.896
HDL-C (mg/dl)	58.17 ± 6.46	53.37 ± 4.87	0.559
TG (mg/dl)	148.84 ± 19.87	200.14 ± 28.66	0.157
LDL-C (mg/dl)	73.04 ± 8.04	69.48 ± 9.72	0.782
Age (Year)	25.82 ± 1.62	24.82 ± 9.72	0.703
BMI (Kg/m^2)	22.66 ± 0.38	22.67 ± 0.33	0.986
Protein	*n =11*	*n =10*	
TP (g/dl)	6.33 ± 0.43	5.83 ± 0.34	0.371
Albumin (g/dl)	3.30 ± 0.20	3.05 ± 0.20	0.391
Globulin (g/dl)	3.06 ± 0.35	2.78 ± 0.40	0.594
Albumin/Globulin	1.32 ± 0.30	1.36 ± 0.21	0.438*
Age (Year)	25.82 ± 1.62	25.30 ± 2.17	0.848
BMI (Kg/m^2)	22.66 ± 0.38	22.62 ± 0.36	0.935

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index

Table 28: Comparison of biochemical characteristics of malaria infected and uninfected women within BMI 25.0 ï 29.9

Variables	**Infected**	**Uninfected**	**ρ value**
Lipid	*n =17*	*n =14*	
TC (mg/dl)	165.38 ± 6.72	154.94 ± 9.03	0.352
HDL-C (mg/dl)	69.72 ± 4.99	75.56 ± 5.15	0.425
TG (mg/dl)	160.60 ± 17.71	151.51 ± 18.06	0.724
LDL-C (mg/dl)	59.67 ± 7.64	49.13 ± 6.06	0.304
Age (Year)	30.47 ± 1.39	25.00 ± 1.75	**0.019**
BMI (Kg/m^2)	27.22 ± 0.36	27.28 ± 0.30	0.903
Protein	*n =17*	*n =16*	
TP (g/dl)	6.11 ± 0.34	5.82 ± 0.35	0.570
Albumin (g/dl)	3.19 ± 0.14	3.52 ± 0.17	0.133
Globulin (g/dl)	2.86 ± 0.34	3.23 ± 0.37	0.189
Albumin/Globulin	1.56 ± 0.24	2.21 ± 0.37	0.171*
Age (Year)	30.47 ± 1.39	24.63 ± 1.54	**0.008**
BMI (Kg/m^2)	27.22 ± 0.36	27.28 ± 0.30	0.903

**significant level determined by Mann Whitney U test, others by Independent Student t test.*
n = number of samples examined, TP = total protein, TC = total cholesterol

Table 29: Comparison of biochemical characteristics of malaria infected and uninfected women of BMI Ó30

Variables	**Infected**	**Uninfected**	**ρ value**
Lipid	*n = 9*	*n =10*	
TC (mg/dl)	152.50 ± 7.61	197.10 ± 15.52	**0.023**
HDL-C (mg/dl)	53.33 ± 8.19	73.25 ± 5.67	0.058À
TG (mg/dl)	172.01 ± 23.79	130.74 ± 23.52	0.235
LDL-C (mg/dl)	64.30 ± 8.23	97.62 ± 18.23	0.121
Age (Year)	27.00 ± 1.48	32.60 ± 1.16	**0.008**
BMI (Kg/m^2)	34.07 ± 1.12	33.05 ± 0.88	0.468
Protein	*n =9*	*n =10*	
TP (g/dl)	6.28 ± 0.51	7.00 ± 0.22	0.180
Albumin (g/dl)	3.44 ± 0.29	3.54 ± 0.13	0.734
Globulin (g/dl)	2.84 ± 0.38	3.54 ± 0.21	0.148
Albumin/Globulin	1.36 ± 0.18	1.06 ± 0.08	0.884*
Age (Year)	26.88 ± 1.48	32.60 ± 1.16	**0.008**
BMI (Kg/m^2)	33.05± 0.51	33.05 ± 0.83	0.973

**significant level determined by Mann Whitney U test, others by Independent Student t test.*

n = number of samples examined, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index. À*P close to 0.05*

3.5 *Plasmodium falciparum* Density and Maternal Biochemical and Other Obstetrics Characteristics

Figure 19 to 38 represent the outcome of the comparison of the obstetrics characteristics of interest in the malaria infected pregnant women based on the intensity of malaria parasitaemia. The malaria parasite density per micro litre of red blood cells were categorized arbitrarily into densities <20000/µL and >20000/µL. The subdivision resulted in 14 pregnant women with malaria parasite density < 20000/µL for the lipid assay category and 23 with > 20000/µL density in the same category. For the protein assay category 14 persons also had <20000/µL parasite density, while 22 had >20000/µL of RBC.

Figures 19 and 20 show the variation in the plasma lipid and protein of the pregnant women whose biochemical characteristics with reference to malaria parasite density were compared. The mean age and BMI of the women were also shown. From the clustered bar chart, figure 19, the TC and BMI of the pregnant women with malaria parasite density of >20000/µL and <20000/µL were almost the same. The HDL-C level in the <20000/µL parasite load group was less than those with parasite density >20000/µL, but not significantly ($p > 0.05$). The age of those with parasite density <20000/µL was higher than those with density >20000/µL, but the difference was not significant.

In figure 20, the total protein, albumin and globulin level for either parasite densities was almost the same. The albumin to globulin ratio value in the higher parasite density (>20000/µL) was less than that in the other density range. The mean age of the <20000/µL malaria parasite density group was higher though not significant at $p < 0.05$.

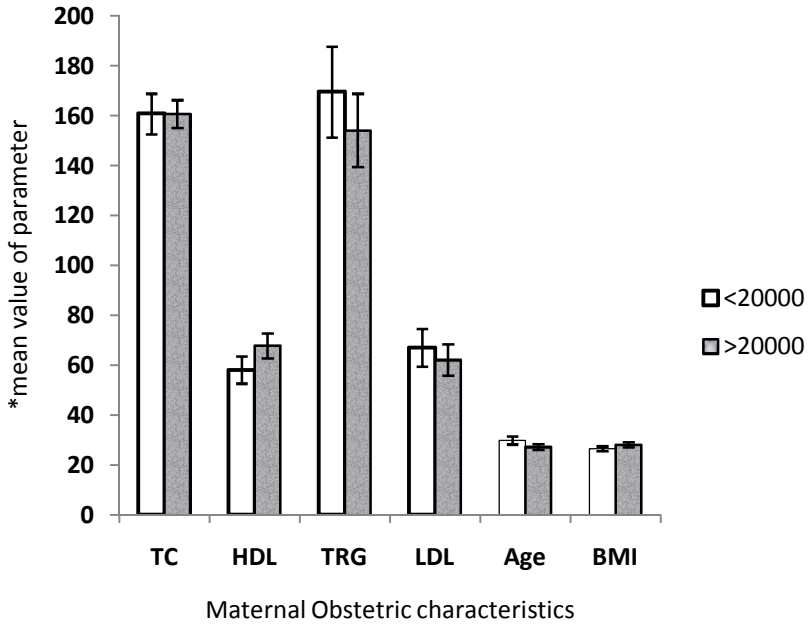

Figure 19: Maternal lipid characteristics at different parasite densities
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

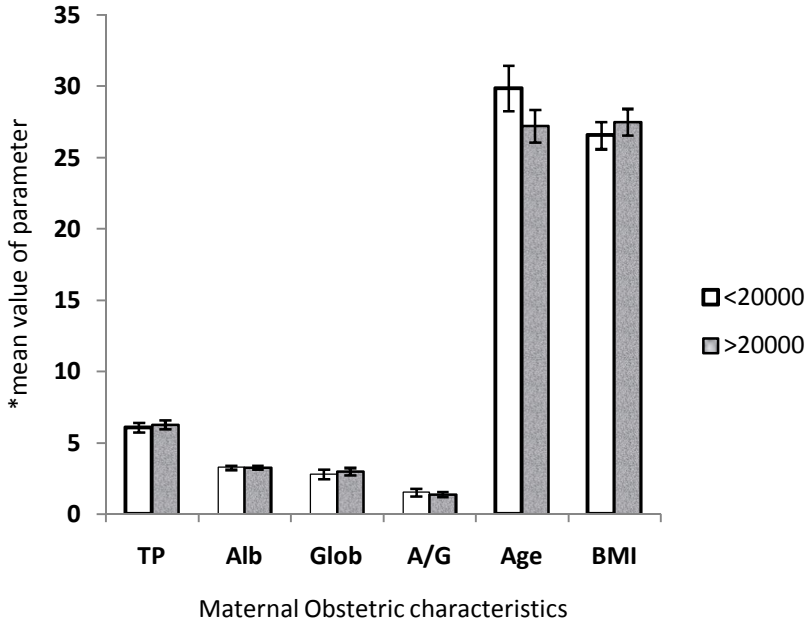

Figure 20: Maternal serum protein and other characteristic at parasite densities
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

3.5.1 *Plasmodium falciparum* density and maternal biochemical and other changes by gravidity, parity and trimester of pregnancy

In this section, the possible effects on maternal biochemical characteristics by the arbitrary parasite densities (<20000/µL and >20000/µL) are presented as compared by gravidity, parity and trimester of pregnancy in the malaria infected.

On the bar chart (Figure 21) comparison among the multigravid women, the mean TC, HDL-C, TG and LDL-C concentration in the two densities of parasitaemia, there were no significant differences observed for all the parameters ($p > 0.05$). The mean ages and BMI were not significantly different as well ($p > 0.05$).

There were no significant differences observed in any of the parameters when the protein profile of the multigravidae by intensity of malaria parasitaemia was compared (Figure 22). The A/G value was, however, lower in the >20000/µL parasite density range as was observed in figure 20. Total protein level was also higher in the >20000/µL group. The difference was not significant ($p > 0.05$).

The plasma lipid characteristics in the multiparous pregnant women were independent on the arbitrary levels of parasite densities, as the variations at all the parameters compared were not significant ($p > 0.05$) (Figure 23). The same trend observed for the protein comparison among the multigravidae (Figure 10) re-occurred in figure 24 for the multiparous women. While there were no significant variation in serum protein indices by the arbitrary densities of parasite, the A/G value remained less in the >20000/µL just as total protein remained higher in the same density category.

When considered by trimester of pregnancy (Figures 25 and 26), the pregnant women with parasite density <20000/µL were by mean age older than those with parasite density >20000/µL. A similar observation of higher concentration for TC, TG and LDL-C levels in the <20000/µL density compared to >20000/µL was made. But the difference in these parameters for either parasite densities was not significant ($p > 0.05$). The total protein, albumin, globulin and A/G values at either parasite densities for the 3rd trimester were very similar.

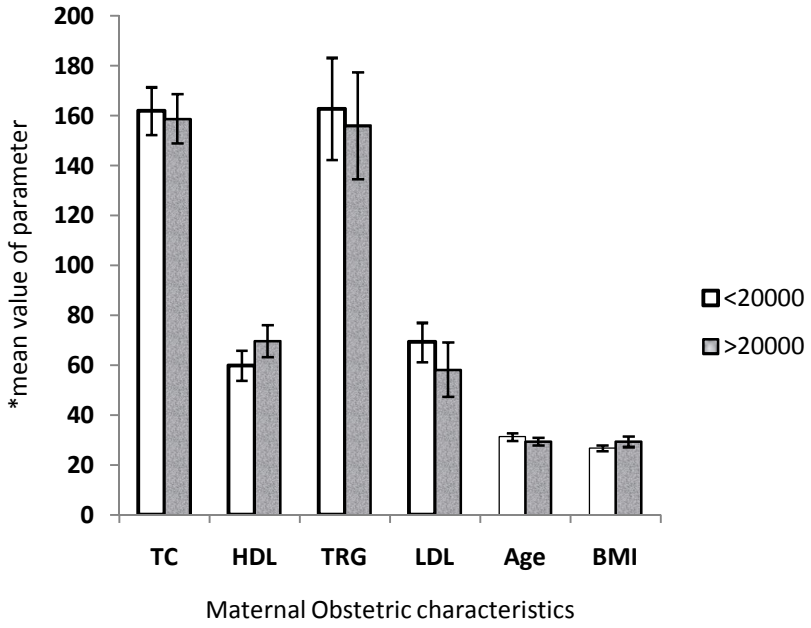

Figure 21: Maternal lipid and other characteristics by parasite density for multigravidae
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

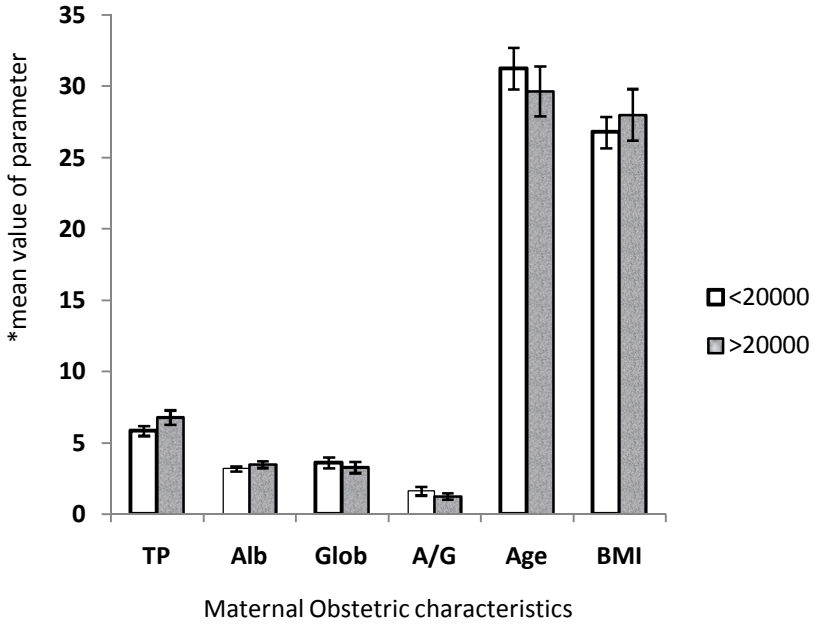

Figure 22: Maternal protein and other characteristics by parasite density for multigravidae
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

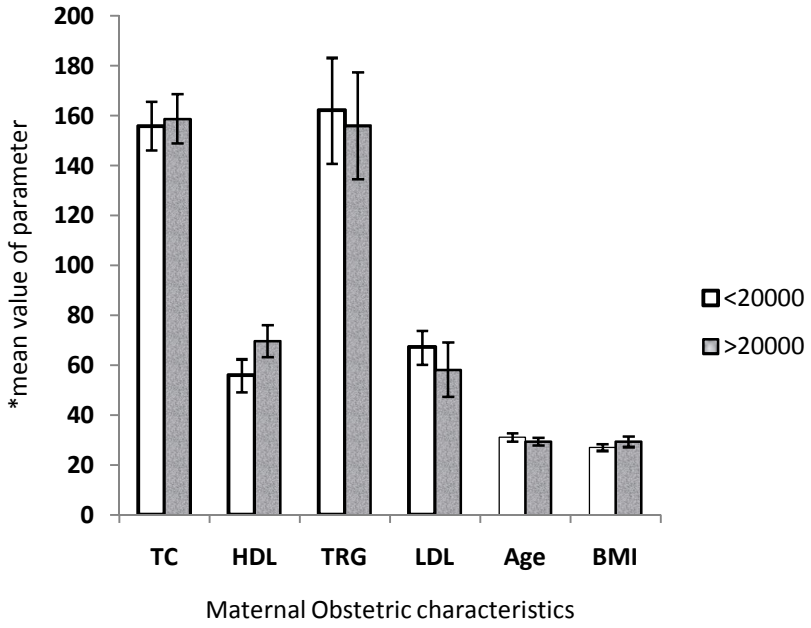

Figure 23: Maternal lipid characteristics by parasite density in multiparity
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

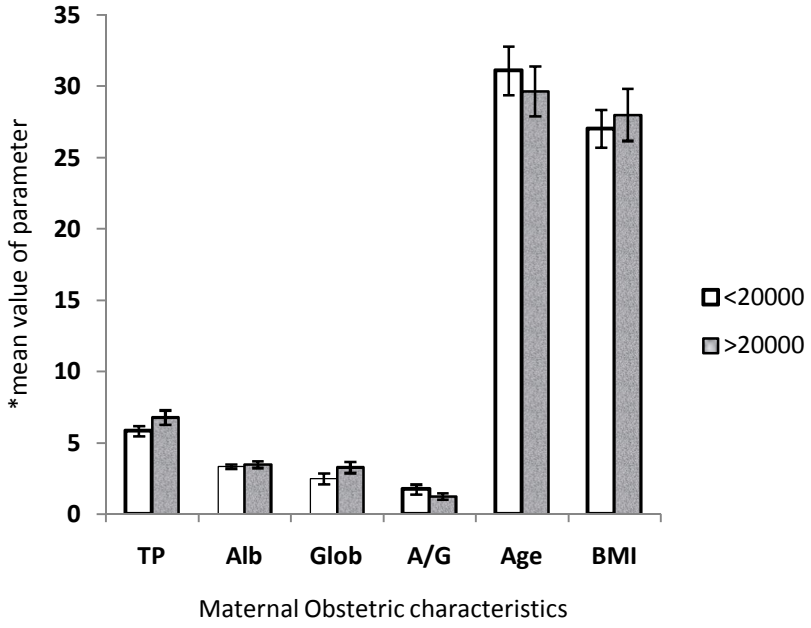

Figure 24: Maternal protein characteristics by parasite density for multiparity
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

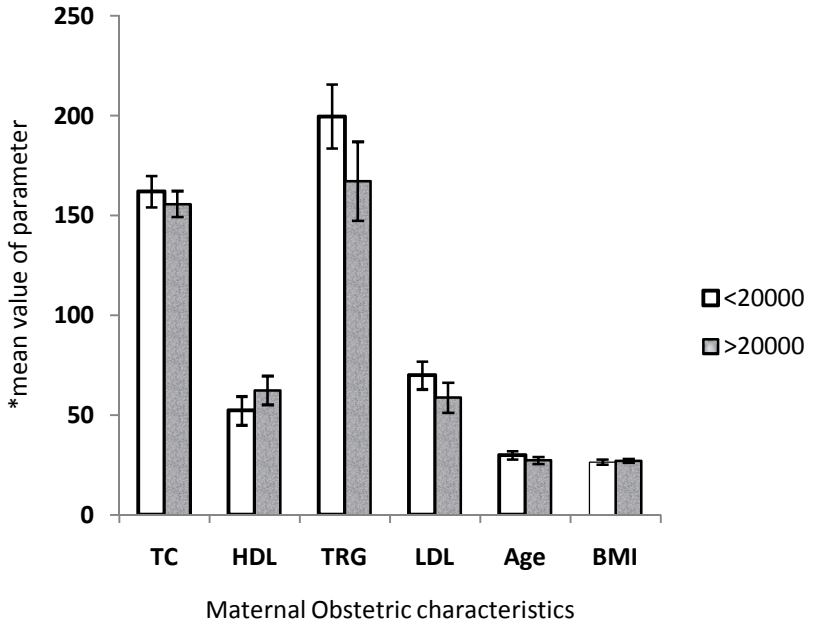

Figure 25: Maternal lipid characteristics by parasite density for 3rd trimester
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

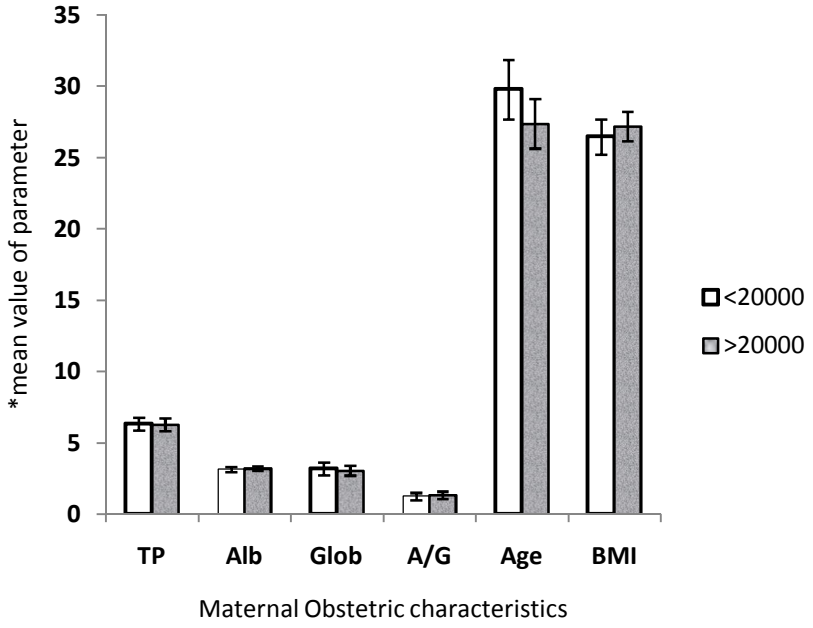

Figure 26: Maternal protein characteristics by parasite density for 3rd trimester
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

The results of the trimesterwise comparison of the biochemical characteristics of the pregnant women with malaria parasite density of >20000/µL to determine whether the high density of parasitaemia could affect the plasma lipid and serum protein level are presented in figure 27 and 28. The TG level remained higher in the women at 3rd trimester and the TC, HDL-C and LDL-C higher in the 2nd trimester. All these variations as well as the mean age and BMI of the groups compared were not significantly different ($p > 0.05$).

This observed difference in significantly higher concentrations of total cholesterol, triglyceride and low density lipoprotein cholesterol in 3rd trimester compared to 2nd trimester elicited further comparisons to determine whether the variation was due to rise in the concentration of these variables in the 2nd trimester as a result of the >20000/µL parasite density or the result of drop in their concentration in the 3rd trimester as a result of the high parasitaemia (Figure 28 to 35).

The concentration of total cholesterol in the uninfected and hyper-infected pregnant women at 2nd trimester of pregnancy did not show any major disparity (Figure 28). But at the 3rd trimester, the total cholesterol concentration in the hyper-infected women was less than that of the uninfected women at all points of comparison (Figure 29). The HDL-C concentration at the second trimester between the infected and uninfected was not significantly different ($p > 0.05$) (Figure 30). At the 3rd trimester, at about 8 points in 13, the HDL-C in the uninfected was higher than that in the high parasitaemia infected women (Figure 31).

The triglyceride level of the women with >20000/µL parasite density at 2nd trimester of pregnancy was less than that in their uninfected counterpart at almost all points of comparison (Figure 32). In a similar comparison for those at the 3rd trimester, the uninfected had a higher triglyceride level at most points of comparison (Figure 33). The LDL-C showed a similar trend as was observed in total cholesterol at either trimester (Figure 34 and 35).

The protein profile of the >20000/µL *P. falciparum* density group in the 2nd trimester and 3rd trimesters were very similar. The TP, albumin and A/G values were only slightly higher in the 2nd trimester with a higher mean age.

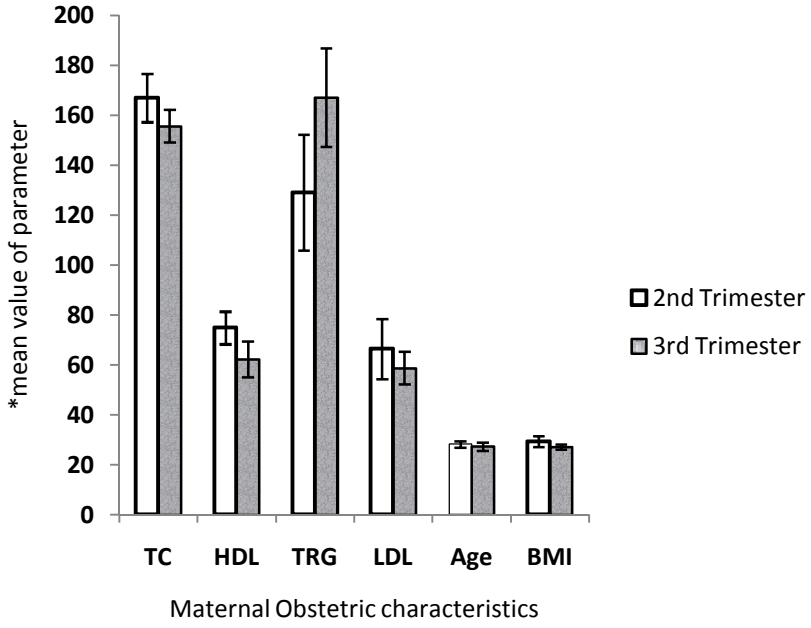

Figure 27: Maternal plasma lipid characteristics between 2nd and 3rd trimesters of pregnancy at parasite density > 20000/µL. * *concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)*^{2}

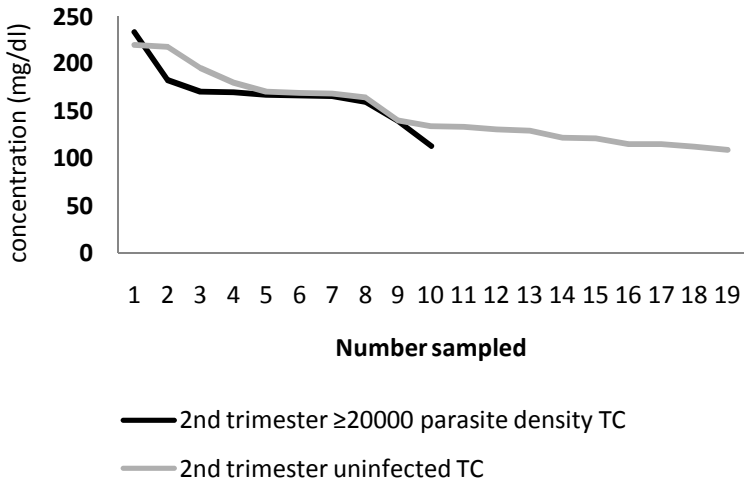

Figure 28: Comparison of total cholesterol concentration of uninfected and > 20000 /µL parasite infected women at 2nd trimester of pregnancy

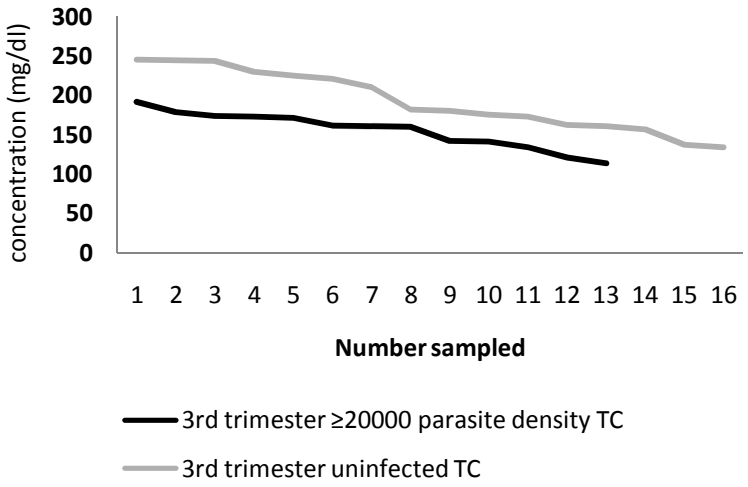

Figure 29: Comparison of total cholesterol concentration of uninfected and > 20000 /µL parasite infected women at 3rd trimester of pregnancy

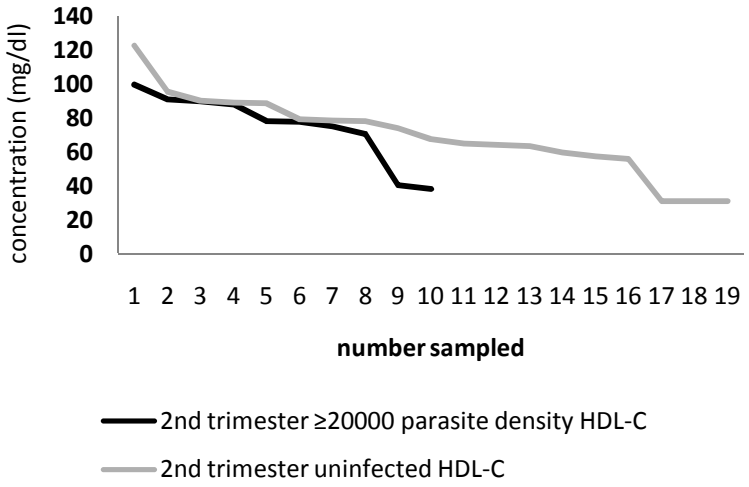

Figure 30: Comparison of high density lipoprotein cholesterol concentration of uninfected and > 20000 /µL parasite infected women at 2nd trimester of pregnancy

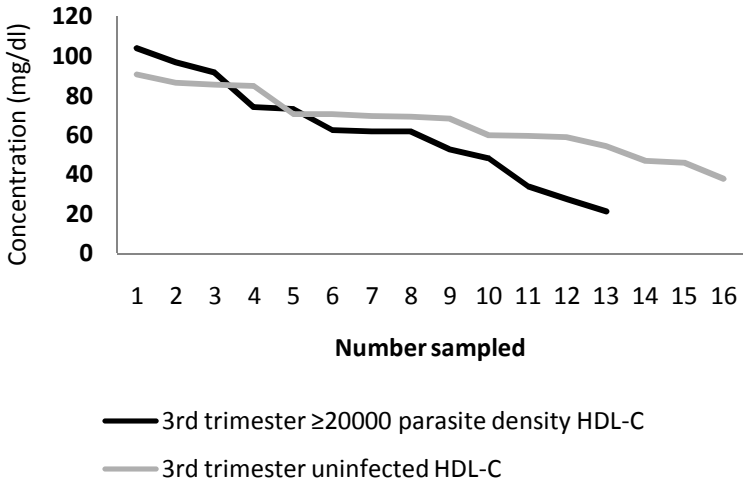

Figure 31: Comparison of high density lipoprotein cholesterol concentration of uninfected and > 20000 /µL parasite infected women at 3rd trimester of pregnancy

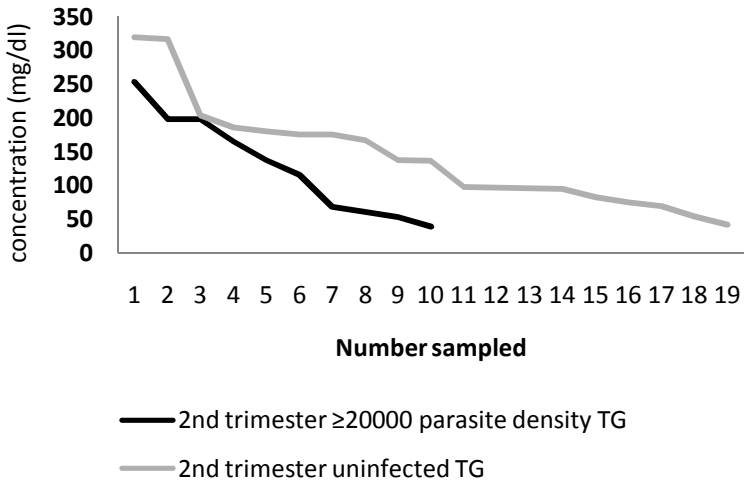

Figure 32: Comparison of triglyceride concentration of uninfected and > 20000 /µL parasite infected women at 2nd trimester of pregnancy

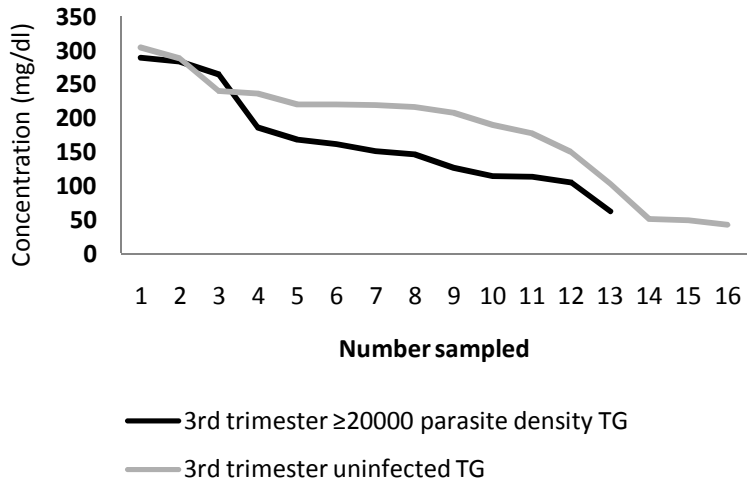

Figure 33: Comparison of triglyceride concentration of uninfected and > 20000 /µL parasite infected women at 3rd trimester of pregnancy

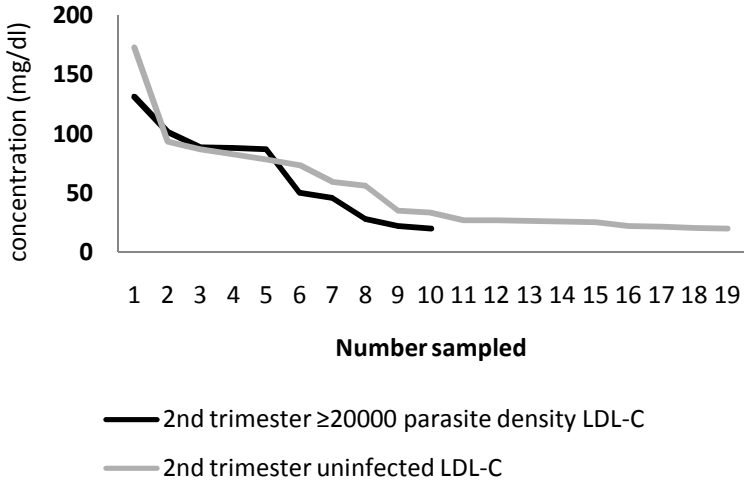

Figure 34: Comparison of low density lipoprotein cholesterol concentration of uninfected and > 20000 /µL parasite infected women at 2nd trimester of pregnancy

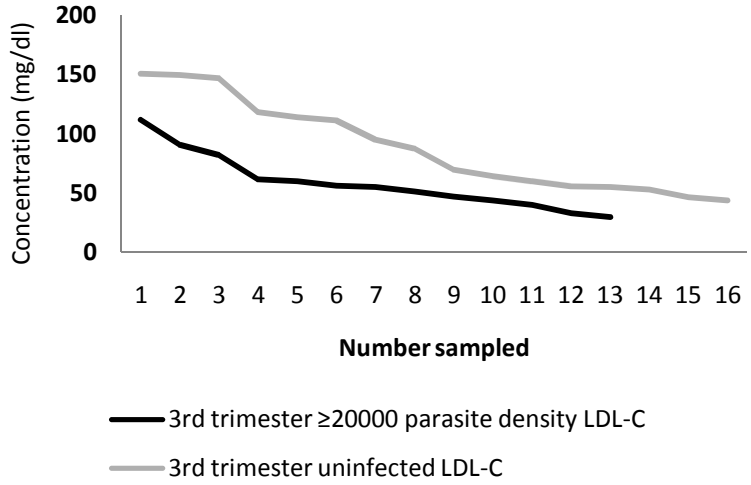

Figure 35: Comparison of high density lipoprotein cholesterol concentration of uninfected and > 20000 /µL parasite infected women at 3rd trimester of pregnancy

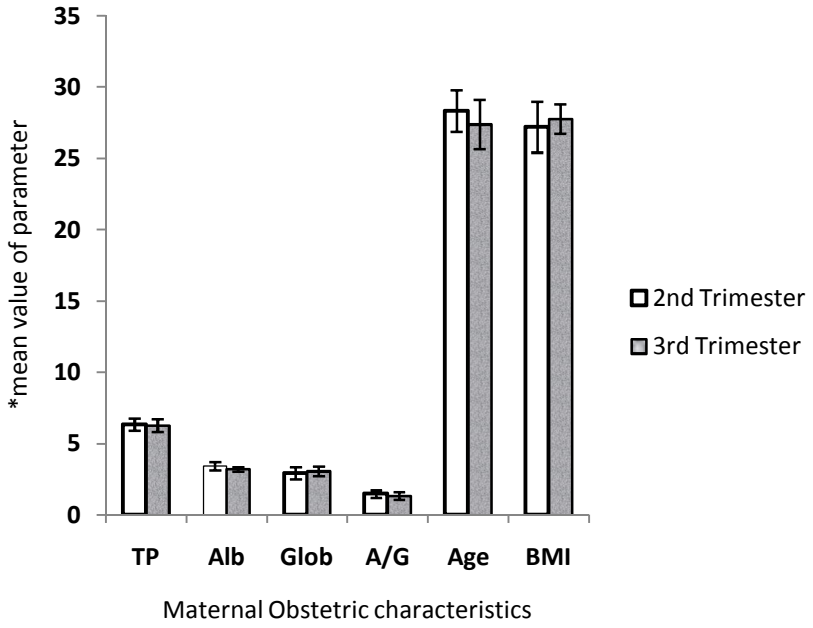

Figure 36: Maternal serum protein characteristics between 2nd and 3rd trimester of pregnancy for parasite density > 20000/µL. * *concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

3.5.2 *Plasmodium falciparum* density and maternal biochemical and other changes by age and body mass index

Total cholesterol and triglyceride concentration was higher in the <20000/µL parasite density group than in the >20000/µL parasite density group for the age group 20 ï 29 (Figure 37). There were, however, no significant differences in all the parameters compared (Ɉ > 0.05). The total protein and globulin level was higher among the 20 ï 29 age group pregnant women with parasite density <20000/µL (Figure 38). The mean age of the <20000/µL parasite density group was significantly not higher than that of the >20000/µL parasite density group (Ɉ > 0.05).

In figure 39 and 40, the plasma lipid and serum protein indices of the pregnant women between age group 30 ï 39 were compared with reference to density of malaria parasite. The HDL-C concentration in the women with malaria parasite density >20000/µL was significantly higher than that observed for those with parasite density <20000/µL (Ɉ <0.05). The TC and TG levels were not significantly higher (Ɉ >0.05) in the >20000/µL parasite density group.

The total protein and albumin level for the comparison made in figure 40 was observed to be higher in women with malaria parasite density >20000/µL. The A/G value was less in the >20000/µL parasite density women in age group 30 ï 39 years. The parameters compared in this figure were not significant variation observed (Ɉ > 0.05).

In order to verify whether the significant variation observed in HDL-C concentration between <20000/µL and >20000/µL parasite density group in figure 39 could have been significantly influenced by age, the lipid indices of pregnant women between 20 ï 29 and 30 ï 39 years but with same parasite density range were compared in figures 41 and 42. Figure 41 showed that the HDL-C concentration in age group 30 ï 39 with parasite density >20000/µL was significantly higher than that in age group 20 ï 29 (Ɉ < 0.05). At the <20000/µL parasite density, shown in figure 42, it was observed that the significantly higher HDL-C in the 30 ï 39 age group was no longer observed, rather, the HDL-C concentration in the 20 ï 29 age became higher.

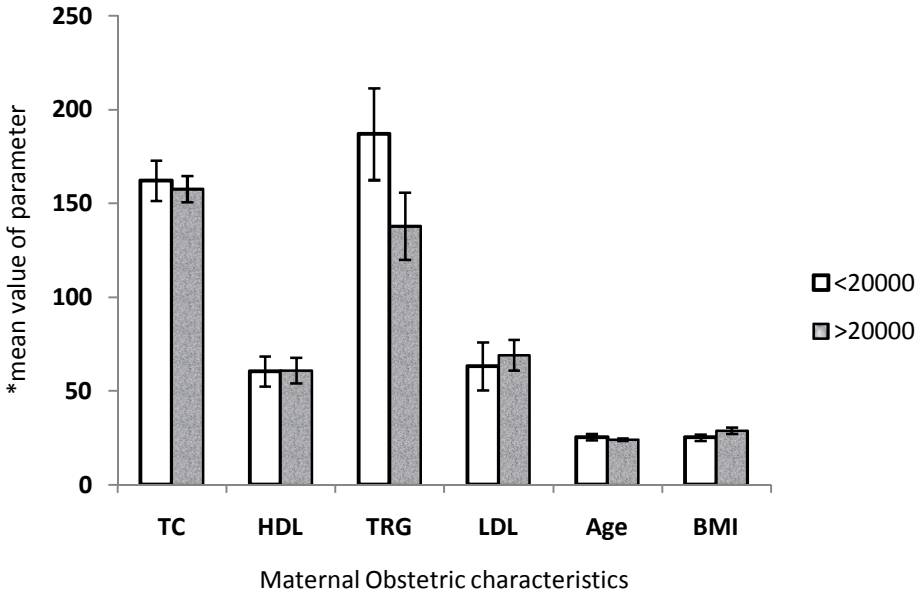

Figure 37: Maternal lipid characteristics by parasite density for age group 20 -29
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

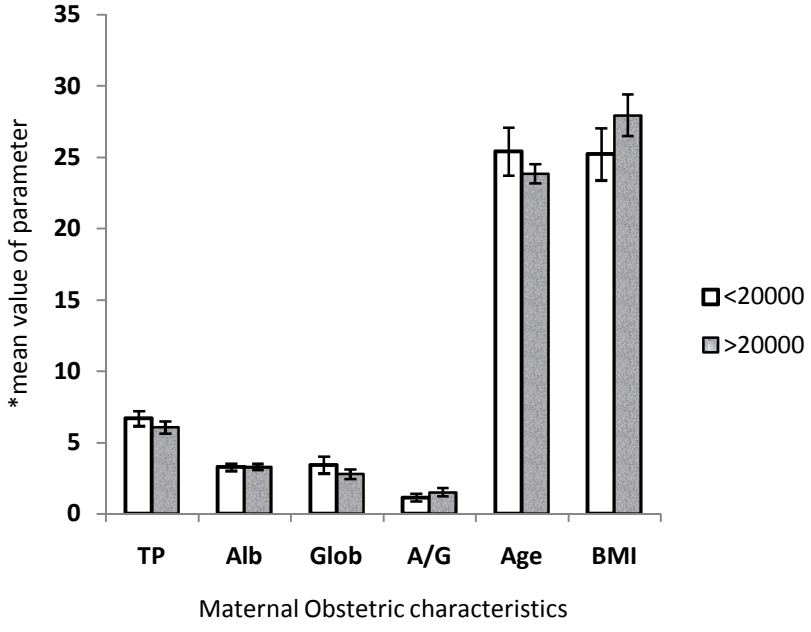

Figure 38: Maternal protein characteristics by parasite density for age group 20 ï 29
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

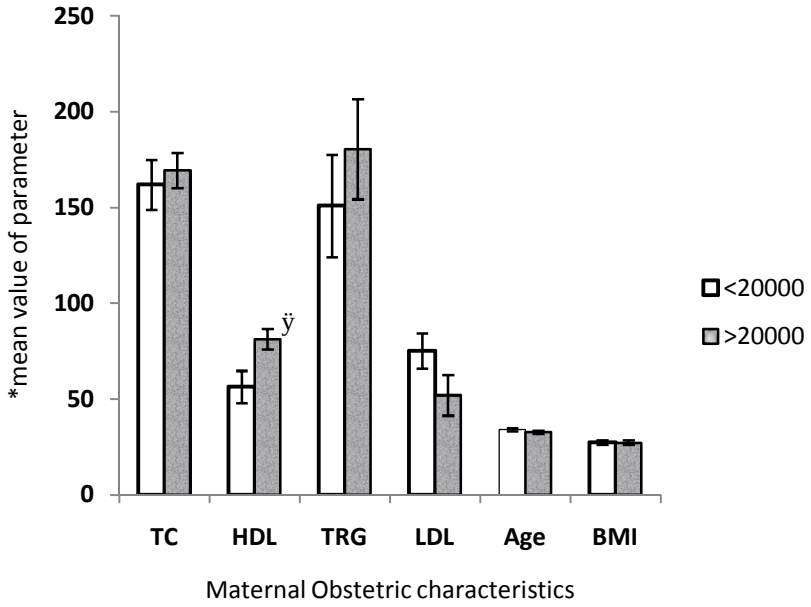

Figure 39: Maternal lipid characteristics by density for age group 30 ï 39

** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

ÿ: *significantly higher (P < 0.05)*

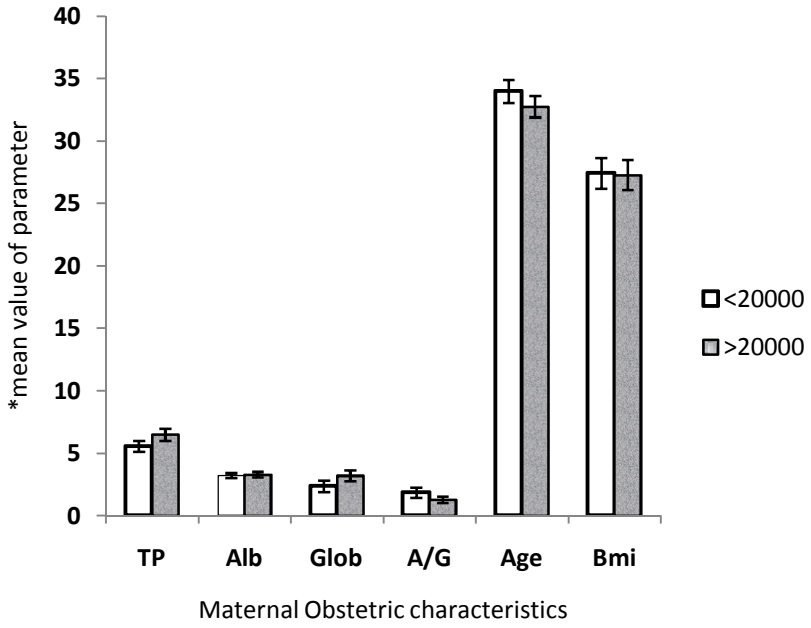

Figure 40: Maternal protein characteristics by parasite density for age group 30 ï 39

** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

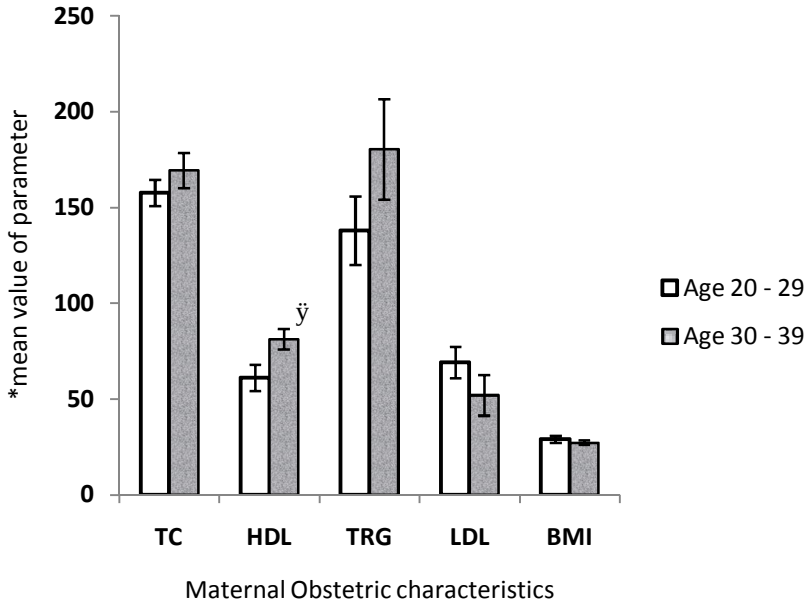

Figure 41: Maternal lipid characteristics between age group 20 ï 29 and 30 -39 at parasite density > 20000/µL. * *concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)*^{2}
ÿ: *significantly higher (P < 0.05)*

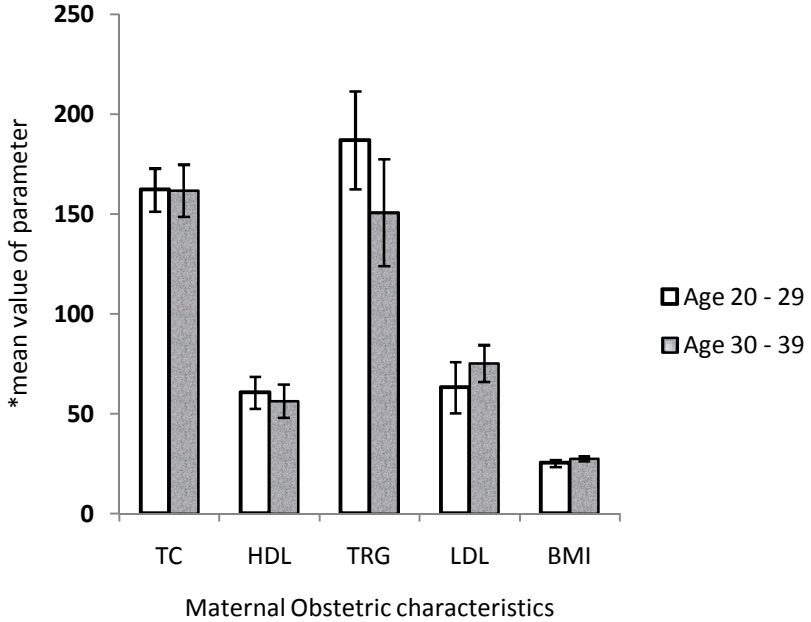

Figure 42: Maternal lipid characteristics between age group 20 ï 29 and 30 ï 39 at parasite density <20000/µL. * *concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)*^{2}

The results of the comparison of the biochemical characteristics within BMI categories by the parasite density are displayed in figure 43 to 46. In figures 43, the TG level in the <20000/µL malaria parasite density group was higher than that in the group with parasite density >20000/µL, but this difference was not significant (ɟ > 0.05). In figure 44, the albumin and A/G values were lower in the >20000/µL malaria parasite density group, but the globulin level in this group was less. The variation was not significant (ɟ > 0.05). The wide difference in mean age between the two groups compared was not significant (ɟ > 0.05).

In the Ó30 BMI category, the lipid profile of the pregnant women with malaria parasite density >20000/µL was not significantly different from those with parasite density <20000/µL (Figure 45). The age and BMI of these groups did not differ significantly (ɟ > 0.05). A very close similarity was noticed for all variables in the comparison of the protein profile by parasite densities within the Ó30 BMI category (Figure 46).

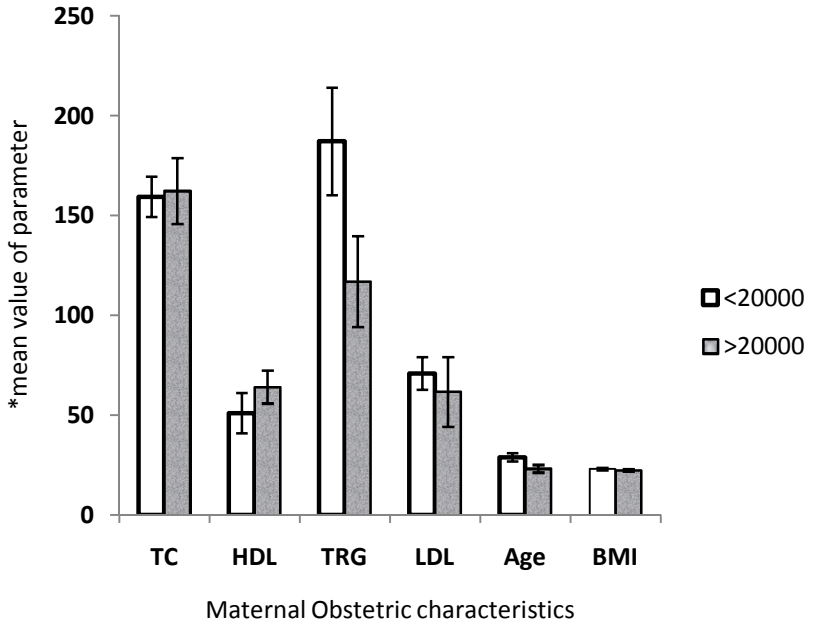

Figure 43: Maternal lipid characteristics by parasite density for BMI group 18.5 ï 24.9
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

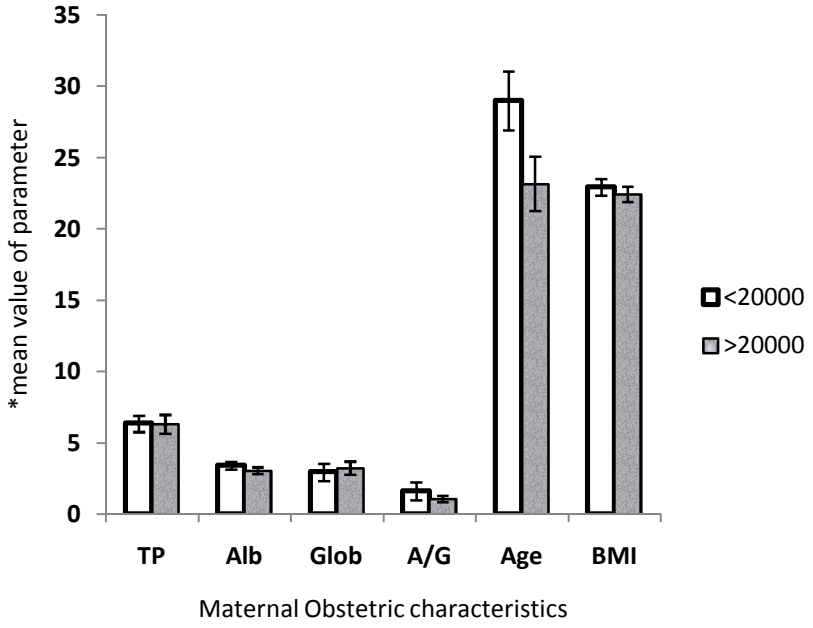

Figure 44: Maternal protein characteristics by parasite density for BMI group 18.5 ï 24.9
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

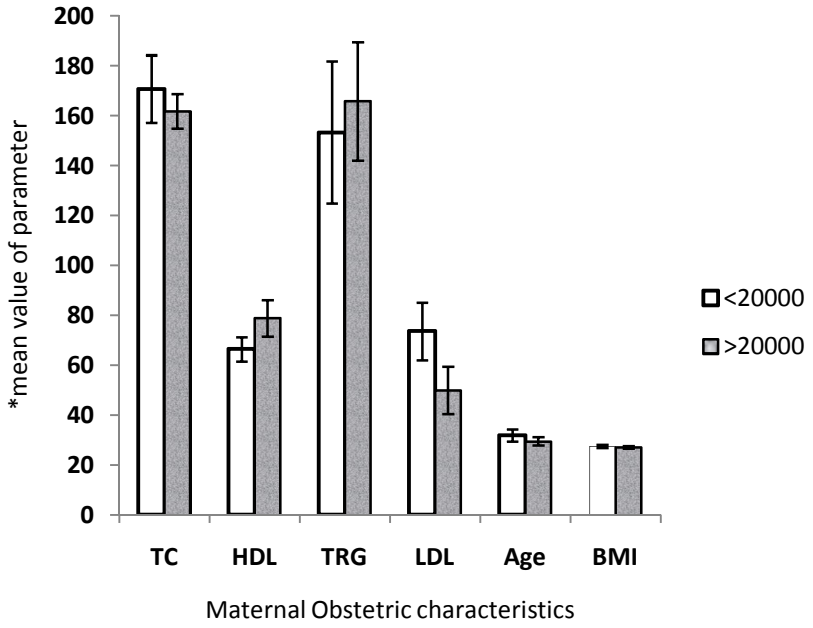

Figure 45: Maternal lipid characteristics by parasite density for BMI Ó30
** Concentration (mg/dl) for lipid, age (yr) and BMI (Kg/m)^2*

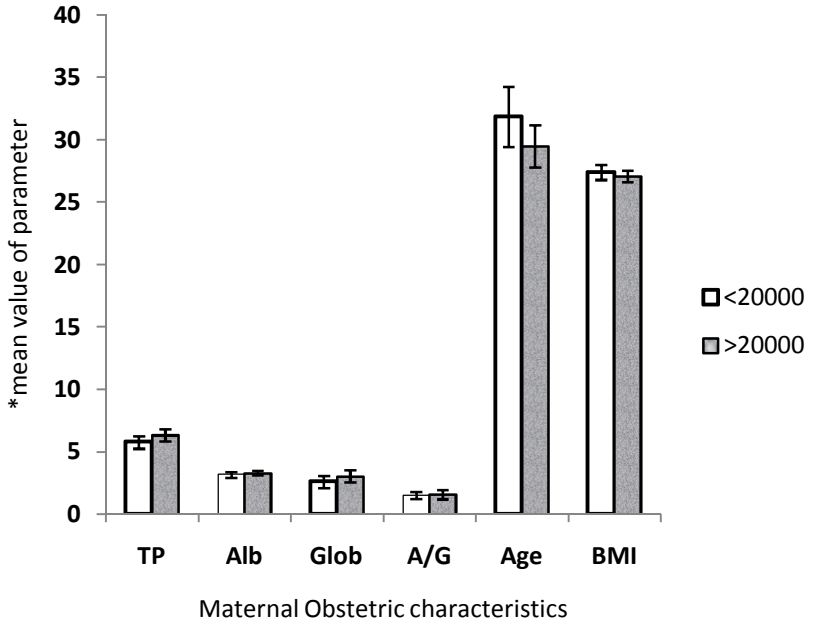

Figure 46: Maternal Protein characteristics by parasite density for BMI Ó30
** Concentration (mg/dl) for protein, age (yr) and BMI (Kg/m)^2*

3.5.3 Correlation and regression of maternal biochemical markers, parasite density and maternal age and BMI

The correlation of the plasma lipid, serum protein, age and BMI of the pregnant women with their age and BMI alongside malaria parasite density among the infected is presented in table 30. From the table, the age of the malaria infected pregnant women had a very significant negative (inverse) relationship with malaria parasite density ($p < 0.01$). The linear relationship between these two parameters is represented by a regression plot (Figure 47). The plot shows a moderately negative linear relationship between age and malaria density in the infected pregnant women {R (%) = 17.7, r = -0.432}. Total cholesterol was significantly correlated with maternal age (r = 0.300) ($p < 0.05$). A similar relationship may exist for HDL-C, though the available data did not support this at $p < 0.05$. The positive but weak relationship{R(%) = 9.0%} between maternal TC concentration and age is shown in figure 50. BMI also had a positive linear relationship with age at pregnancy {R(%) = 11.56, r = 0.330} (Figure 48). Maternal albumin concentration during pregnancy showed a weak positive linear relationship with BMI {R(%) = 9.13, r = 0.302} as it increased as BMI increased (Figure 49).

Table 30: Correlations (r values) between maternal biochemical parameter and parasite density, maternal age and BMI

Maternal Clinical Characteristics	**Parasite Density (/µL)**	**Age (Year)**	**BMI (Kg/m^2)**
	n = 36	*n = 72*	
TC (mg/dl)	-0.113	0.300*	0.179
HDL-C (mg/dl)	0.139	0.226À	0.154
TG (mg/dl)	-0.313À	0.047	-0.202À
LDL-C (mg/dl)	0.026	0.177	0.162
TP (g/dl)	-0.056	0.144	0.168
Albumin (g/dl)	0.054	0.144	0.302**
Globulin (g/dl)	-0.088	0.093	0.023
Albumin/Globulin	0.081	0.095	0.016
Age (Year)	-0.432**	1	0.330**
BMI (Kg/m^2)	-0.208	0.330**	1

***correlation is significant at the 0.01 level (2- tailed). * correlation is significant at the 0.05 level (2-tailed). †Significant level very close to 0.05. n = number examined. TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, TG = triglyceride, LDL-C= low density lipoprotein cholesterol, TP = total protein, BMI = body mass index*

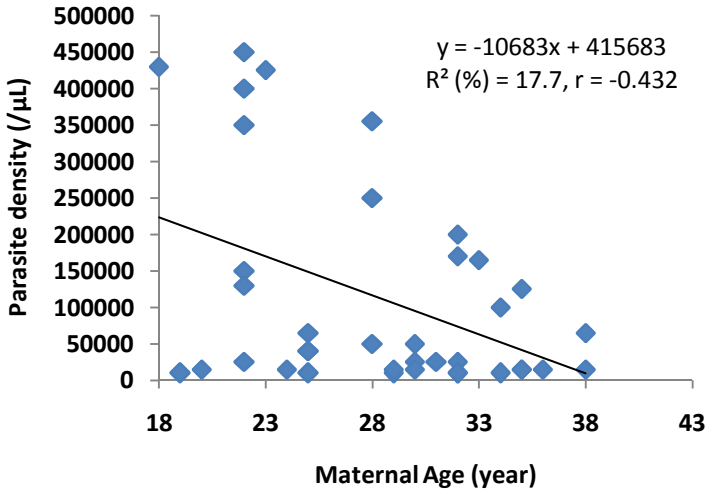

Figure 47: Regression of parasite density against maternal age

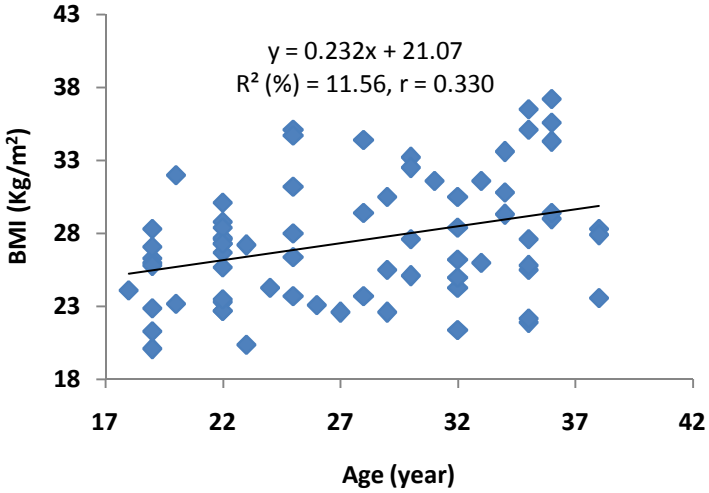

Figure 48: Regression of BMI against Age

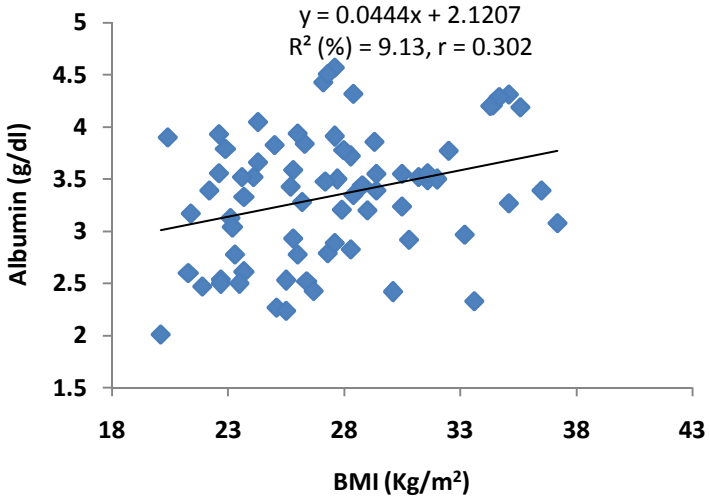

Figure 49: Regression of Albumin against maternal BMI

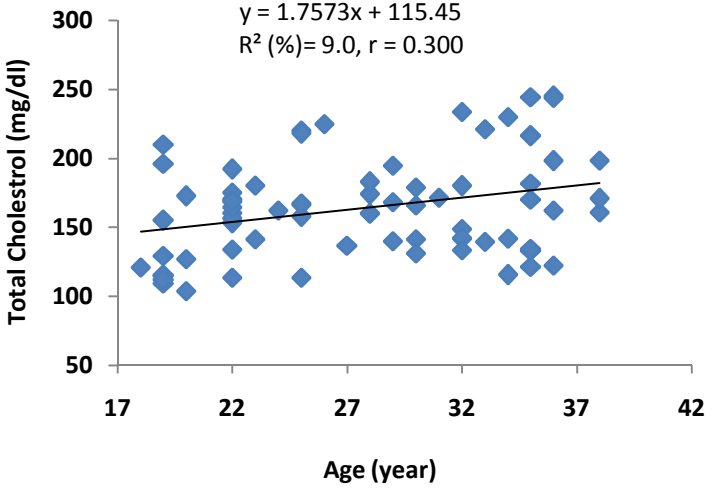

Figure 50: Regression of Plasma total cholesterol against maternal age

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Prevalence and Intensity of Malaria in Igbo-Eze North Local Government Area

From this study on pregnancy-associated malaria and associated biochemical changes and BMI indices in Igbo-Eze North North Local Government Area, Enugu State, Nigeria, the overall prevalence and parasite density 50.7% and 112894.74 ± 22901.73/µL respectively. A similar but slightly higher overall prevalence of 52% was recently reported from Yemetu-Adeoyo, a semi-urban community in Ibadan, Nigeria (Ayoola *et al.*, 2012). Some other studies in different parts of Nigeria have reported a much higher overall prevalence of pregnancy-associated malaria, such as 65.6% in Ebonyi State (Ivoke *et al.*, 2013,), 72% in Osun State (Adefioye *et al.*, 2007) and 88% in Nassarawa State (Alaku *et al.*, 2015). A much lower pregnancy-associated malaria prevalence of 7.7% was reported in Lagos, Nigeria (Agomo *et al.*, 2009) which is rare in Nigeria where *P. falciparum* endemicity and associated morbidity is a major public health challenge. Ofori *et al.* (2009) also reported a low overall prevalence of *P. falciparum* pregnancy-associated malaria of 19.7% in Ghana.

The relatively high overall prevalence of pregnancy-associated malaria reported in the present study may be attributed to several factors that encompass host-parasite relationship (Faik *et al.*, 2009), malaria parasite and vector behaviour (Cator *et al.*, 2015; Churcher *et al.,* 2015), parasite host behaviour and physiology (Cai *et al.*, 1995; CDC, 2012; Cator *et al.*, 2012; 2015), host and parasite environment (Cai *et al.*, 1995; Nkuo-Akenji *et al.*, 2006) and available medical facilities and usage. The outcome of the investigation of an aspect of the human host behaviour toward malaria, the non-usage of intermittent preventive treatment and poor usage of mosquito bed net probably contributed. Though 49.3% of the pregnant women examined had insecticide treated bed net, only 32.0% used it. While these malaria preventive procedures were not strongly adhered to, strong monitoring in the form of extremely subsidized routine rapid diagnostic tests (RDTs) for malaria was a common practice at the District Hospital, Enugu Ezike. The diagnosis was, however, subject to the pregnant womanôs consent. As an environmental factor, the areas in close proximity to homes may have contributed to this relatively high prevalence observed. The people in most places within the study area live in close proximity to bushes sometimes with

pools of water which may provide breeding sites to mosquitoes. The influence of season may be another contributing factor (Charlwood *et al.*, 2000; Nkuo-Akenji *et al.*, 2006). This is especially as sample collection encompassed the rainy season (August to October) and early dry season (November to December) when mosquito population and malaria transmission is usually high (Lindsay *et al.*, 1991; Awodu and Enosolease, 2003; Odongo-Aginya *et al.*, 2005).

The mean parasite density per microlitre of red blood cell of 112894.74 ± 22901.73 is high (PARN, 2009). This conclusion on high parasite overall density may have to be taken with great deal of caution. As only 16 (42.1%) of the 38 infected person had parasite density greater than 50 000/µL (maximum for intermediate level between high and moderate [PARN, 2009]) and 18 (47.4%) had parasite density below 30 000 /µL (maximum for moderate level of parasitaemia [PARN, 2009]). The high *P. falciparum* parasitaemia level observed in 42.1 % of the PAM cases may be attributed to delayed diagnosis and treatment (WHO, 2012; CDC, 2015), and inconsistent and delayed antenatal visits. This is more so as 37 (49.3%) of the subjects of this study were on their first antenatal visit even though 35 (95%) out of these 37 were already passed the first trimester. Where effective diagnosis exists as was the case at the District Hospital Enugu-Ezike, delayed attendance for antenatal check-ups and non-commitment to voluntary malaria diagnosis and treatment may be contributing factors to high malaria prevalence. This appears to be the case as 18 (47.4%) of the people infected were on first antenatal visit. When these two factors were considered, it was observed that the mean parasite density in those that had 2 or more antenatal visits for the same pregnancy at the same clinic before the commencement of this study, had a higher *P. falciparum* parasite density than those visiting for the first time. This suggests that non-commitment to regular diagnosis and treatment may be the major contributor to the observed high parasitaemia level. Also, those on first antenatal visits were more unlikely to own and use mosquito bednet which is a predisposing factor to pregnancy-associated malaria (Clarke *et al.*, 2001).

Maternal immunologic state may contribute also to high parasite density (Smereck, 2011). Chandrasiri *et al.* (2014) studied the immunologic state of pregnant women and its relationship to severe malaria complications and concluded that among Sudanese pregnant women, cases of severe PAM correlated with lack of pregnancy-specific malaria immunity.

Pregnancy-associated malaria caused by *P. falciparum* is known to be a major concern among primigravidae and secundigravidae (Jamieson *et al.*, 2006; Maestro-Carmona-Fonseca, 2014). In most cases, primigravidae which are the more threatened, fall under nulliparity category and the secundigravidae under the primiparity category. This gravidity and parity dependent virulence is blamed on the absence in the primigravidae (or nulliparous in most cases), or poorly developed in secundigravidae (or primiparous in most cases), of immunity to *P. falciparum* pregnancy-associated variant surface antigen (VSA_{PAM}), unlike the multigravidae (or multiparous in most cases) where such exist (Fried and Duffy, 1996; Nielson *et al.*, 2007). Such absence of immunity possibly predisposes the pregnant primigravid woman to hyperparasitaemia. As observed from this study, *P. falciparum* parasitaemia was significantly higher in the primigravidae ($P < 0.01$) and nulliparous ($P < 0.01$) compared to their multigravid (and primigravid) and multiparous (and primiparous) counterparts respectively.

This high level of parasitaemia in pregnancy where immunologic response is known to shift towards the humoral aspect in order to accommodate the foetus, induce pathologic and physiologic stress with deleterious consequences on the gravidae and her foetus. High maternal blood *P. falciparum* parasitaemia and subsequent cord blood localization of *P. falciparum,* and the resultant shift of immune response from the humoral arm to the cell-mediated leads to antagonistic response against foetus and subsequent spontaneous abortion of foetus and pre-term labour (Jamielson *et al.*, 2006; Maestre and Carmona-Fonseca, 2014). Low-birth weight, intrauterine growth retardation, neonatal death and still birth (Saba *et al.*, 2008; Schantz-Dunn and Nour, 2009; and Ayoola *et al.*, 2012) may characterize this observed high parasitaemia in the primigravidae and secundigravidae. The development of some level of immunity in the secundigravidae or primiparous women (Jamielson *et al.*, 2006), probably contributed to the lower parasite density. But a combination of the primigravidae and secundigravidae (primi+secundigravidae) still showed a higher parasite density compared to the multigravidae.

Another cardinal aspect of the observation of *P. falciparum* pregnancy-associated malaria from this study is the highest prevalence of malaria infection among the multigravidae (57.5%) compared to the primigravidae (38.1%) and secundigravidae (50.0%) and among the multiparous (56.4%) women compared to that among the nulliparous (46.2%) and primiparous (40.0%) counterpart. Though these disparities were not significant at $\text{ɟ} < 0.05$, it is a pointer toward

asymptomatic response among the multigravid and multiparous women living in an area where *P. falciparum* is endemic. This higher cases among the aforementioned categories possibly resulted from none diagnosis and treatment that characterize asymptomatic state (Høgh, 1996). Yahaya *et al.* (2009) and Taura and Oyeyi (2009) reported higher prevalence of pregnancy-associated malaria in primigravidae compared to multigravidae. This implies that other factors apart from gravidity affect pregnancy-associated malaria prevalence.

The contribution to the overall prevalence of pregnancy-associated malaria observed in the third trimester (63.9%) compared to the second (36.1%) may have arisen from progressive immunomodulation in pregnancy. During pregnancy, maternal systemic circulatory levels of IL-4, IL-6, IL-10 and IL-13 (Th2 or humoral response promoting cytokines) is believed to increase progressively while the plasma concentration of IL-1Ŭ, IL-1ƀ, Il-2, IL-12 and INF-ɔ (Th1 or cell-mediated response promoting cytokines) decrease (Shimaoka *et al.*, 2000; Sykes *et al.*, 2012). The decrease in Th1 cytokines become significant in the third trimester compared to the first; this decline is directed at sustaining the foetus. This shifting of immune response towards the humoral aspect, though advantageous to the foetus sustenance, it is not efficient in *P. falciparum* clearance (Moureau and Chauvin, 2010). This increased retention of malaria parasite characterizing this cell-mediated-humoral response shifting, may be the reason for the higher third trimester prevalence. This view places a question mark as to why the parasite density was not significantly higher at the third trimester. Studies did not exclude humoral response in the control of malaria parasitaemia, in fact it is important (Mayxay *et al.*, 2001; Rogerson *et al.*, 2007). It appears as though humoral and cell-mediated response complement each other in the control of *P. falciparum*. The gradual loss of this synergistic activity in post first trimester probably led to the higher parasitaemia observed in the third trimester.

The non-significantly higher (ɟ > 0.05) prevalence and the significantly higher (ɟ < 0.05) parasite density observed in the <20 ï 29 age group compared to the 30 ï 39 can best be interpreted with reference to the very high significant variation (ɟ < 0.000) in gravidity and parity by age reported in this study. The same reason accounts for the negative linear relationship observed between age and parasite density. People of younger age were more likely to fall under the primigravid and nulliparous categories and hence it follows that they suffer similar intensities of malaria parasitaemia as the primigravid and nulliparous women. Hence the reasons for the

observed significantly higher malaria parasite density in < 20 ï 29 are already accounted for in the discussion on parasite density by gravidaity and parity. The absence in the primigravidae and poorly developed in secundigravidae of immunity to *P. falciparum* is very likely the chief cause of the significantly higher parasitaemia in the <20 ï 29 age group. Why the above reasons account for the high density, the reason for the higher prevalence in < 20 ï 29 may be entirely due to chance.

Parasite prevalence and density was observed not to exhibit significant variation (ɟ > 0.05) by BMI categories. As a predictor of maternal nutritional state, BMI may determine maternal health status and predisposition to disease (Buys *et al.*, 2014). While the view that under nutrition predispose to malaria infection may be cautiously taken (Alexandre *et al.*, 2015), to link BMI to nutrition and establish an inverse relationship between BMI and malaria is even much more controversial. This is especially so as there are many confounders to such assessment. It is no doubt such BMI predisposing prevalence is not supported by the result of this study. The higher intensity of parasitaemia in the 18.5 ï 24.9 (normal) category may be associated with lower BMI of the younger people in this study compared to their older counterparts. But Alexandre *et al.* (2015) reported a reduction in children BMI due to malaria. Therefore, the highest parasite density at the lowest BMI category reported in the present study may be considered as resulting from the tendency of malaria parasite density to reduce sufferersô weight or BMI.

Looking at the outcome of the comparison of malaria status of the pregnant women in relation to communities and villages and town, the similarities observed possibly resulted from similarity in antenatal care provided the pregnant women from the different communities. Repeated weekly attendance to clinic for antenatal care and regular counselling as was observed in the course of the study may have contributed significantly to this outcome.

4.2 Biochemical and Other Characteristics during Pregnancy

Plasma biochemical such as lipids and proteins are important diagnostic parameters. The assessment of these parameters is possible predictors of organismal physiological, immunological, anatomical and pathological state. Diagnostic significance of the plasma or serum biochemical is hinged on the skewing of these parameters away from expected range by

physiological and pathological stressors (Anderson and Anderson, 2002). Pregnancy is a physiological state known to significantly modify the biochemical architecture of the plasma biochemistry (Costatine, 2014). These pregnancy-associated alterations are important for foetal sustenance.

Body mass index (BMI), another predictor of organismal state relies on morphometric characteristic. As a factor employable in the assessment of human health status as it relates to nutrition and predisposition to cardiovascular disturbances, it is relevant in the assessment of maternal health. As a predictor of weight gained during pregnancy, Adair (2007), Ludwig *et al*. (2013) and Almond and Currie (2011) have linked BMI to neonatal survival, neonatal birth weight, and childhood and adulthood height.

Normal pregnancy is associated with progressive increase in the level of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) (Diareme *et al.*, 2009; Ayoola *et al.*, 2012; Al-Tawil, 2013). From the present study, while it was observed that there was no significant rise or decline in TC, HDL-C, TG and LDL-C by gravidity and parity, progressive rise from 2nd trimester to 3rd trimester in these parameters was observed. In the present study, TC, TG and LDL-C were significantly higher ($p < 0.05$) at the third trimester of pregnancy compared to the second. HDL-C was much higher in the third trimester as well ($p = 0.061$). This observation further justifies recent researches and recommendations (Nordestgaard *et al.*, 2007; Sidhu and Naugler, 2012; Ontario Association of Medical Laboratories [OAML], 2013; White *et al.*, 2015) that it may be unnecessary to fast before lipid test. The progressive rise in plasma lipid during pregnancy is associated with positive (Diderholm *et al.*, 2005) and negative (Vrijkotte *et al.*, 2012) consequences for the pregnant woman and her foetus. De *et al*. (2006) and Vrijkotte *et al.* (2012) observed that non-fasting TG level was linearly associated with pregnancy-induced hypertension (PIH), preeclampsia and induced preterm labour.

The progressive rise in plasma lipid concentration as observed in this study and characteristic of second and third trimester of pregnancy is associated with increased adipose tissue lipolysis. Accumulation of fat in the adipose tissue occurs in the first trimester of pregnancy; this is accompanied by post-first trimester increased lipolysis which peaks at the third trimester (Diderholm *et al.*, 2005). Hyperlipidaemia accompany the increased lipolysis, and this provides

substrate for energy metabolism in the maternal system sparing glucose for the foetus (Diderholm *et al.*, 2005). Intrauterine growth restriction is possibly associated with reduced rate of lipolysis or glycerol production (Diderholm *et al.*, 2006). Similarly, lipoprotein receptors and fatty acid binding protein in the placenta allow the transfer of long chain polyunsaturated fatty acids (LC-PUFAs) to the foetus during late pregnancy when foetal growth is at its peak (Herrara *et al.*, 2006).

Serum total protein (TP) and albumin have been established to undergo progressive change during pregnancy. Reports about progressive changes in globulin have not been consistent. Where albumin and/or globulin undergo changes, invariably, albumin to globulin ratio (A/G) undergoes variations as well. Serum total protein concentration fall during pregnancy. The reduction take different course: it may decline continuously, or plateau after the second trimester or may rise prior to delivery after initial decline (Joseph *et al.*, 1978). The increased plasma water concentration and the resultant plasma dilution during pregnancy (Constantine, 2014) which has an inverse relationship with total protein concentration (John *et al.*, 2010) has been suggested as a possible cause of the decline in total protein concentration (Joseph *et al.*, 1978). The period of rapid growth of the foetus is accompanied by the recruitment of protein from maternal circulation (Lewis *et al.*, 2010).

From the present study, the total protein level in either trimester (second and third) was not significantly different; it appeared to plateau. The slight rise observed in the third trimester may be ascribed to possible rise that accompany the pre-delivery serum total protein level (Lewis *et al.*, 2010). This is more so, as 12 out of the 38 (31.6%) women examined in this category were already at their 9^{th} month of pregnancy. The albumin level in the present study declined slightly from the second to the third trimester. This observed decline is consistent with prior observation from other studies (Joseph *et al.*, 1978; John *et al.*, 2010). Albumin concentration is known to decline progressively during pregnancy as a result of 40 ï 50% plasma volume increase and haemodilution during pregnancy (Ciliberto *et al.*, 1998).

The variation in total serum globulin during pregnancy is controversial, while some studies have reported non-significant progressive rise (Jemikalajah and Adu, 2015) and other have reported oscillation significant at some point (Adedeji *et al.*, 2012), others have contrastingly reported non-significant decline (Al-Tawil, 2013). These changes may be associated with the response of

subsets of globulin at various physiological states. Globulin level in this study was not significantly higher in the third trimester. This is in line with largely observed condition (Joseph *et al.*, 1978; Jemikalajah and Adu, 2015). The progressive rise in globulin and decline in albumin accounts for the less A/G value in the third trimester compared to the second.

Data on variation of maternal plasma lipid by gravidity or parity at pregnancy is extremely lacking. Though data was available online on lipid profile of primigravid and nulliparous pregnant women with preeclampsia; data was grossly lacking for comparison of lipid characteristics at pregnancy between primigravid, secundigravid and multigravid pregnant women. However, Mankuta *et al.* (2010), in a 6-year cohort study examined the lipid profile in consecutive pregnancies. Even though, the study design differed from the present which is cross-sectional, the outcome of their study is instructive. They reported a significant rise in TC ($P < 0.0001$), LDL-C ($P < 0.0001$), TG ($P < 0.0001$) and HDL-C ($P < 0.0001$), and a within one year post partum return to normalcy in TC and LDL-C (Mankuta *et al.*, 2010). According to their study, LDL-C level continued to decline attaining a level lower than the pre-pregnancy level two to three years post partum, while the HDL-C level plateau and do not decline further one year post partum; it level was lower in subsequent pregnancies (Mankuta *et al.*, 2010). Integrating the observation by Mankuta and co. (2010) with this present study, the non-significant variation in comparison of lipid profile by gravidity and parity is justified. The higher level of TC observed in the secundigravidae and multigravidae is justified as significant difference exists between the ages at the gravidities and between the nulliparous and multiparous women. This observation taking together with the fact that age correlated significantly with TC concentration, in the pregnant women in this study resolves that problem. The significant difference observed in LDL-C between primigravidae and secundigravidae is not accounted for by age. The significant variation in LDL-C probably arose as an attribute of the sampled population.

Weight (or BMI) gain or reduction induces plasma lipid changes. Cordero-MacIntyre *et al*. (2004) reported an association between weight losses in postmenopausal women and the reduction in TC, TG and LDL-C. Dattilo and Kris-Etherton (1992) in using a meta-analysis established a highly significant association between weight reduction and TC, LDL-C and TG. Plasma protein profile is dependent on age (Ignjatoric *et al.*, 2011; Monteiro *et al.*, 2014) and obesity (Tchnernof *et al.*, 2002). A linear association between age and BMI as reported in this

study has long been observed (Miida *et al.*, 1996; Wilson *et al.*, 2002). In the assumption that point BMI and age at pregnancy are predictors of plasma lipid and protein indices in the assessment of maternal biochemical characteristics during pregnancy, as a check on malaria induced changes, if any, and as a check on non-fasting plasma lipid characteristic, a positive linear relationship {R^2(%) = 11.56, r = 0.330} between BMI and age of the pregnant women in this study was similarly obtained. A positive correlation between TC and age was similarly reported by Gostynski *et al.* (2004) among males and non-pregnant females. But unlike report by Wakabayashi (2004), correlation between BMI and HDL-C was absent in this study.

4.3 Pregnancy-associated Malaria and Plasma Biochemical Changes

Non-pregnancy and pregnancy associated malaria is believed to induce changes in plasma biochemical characteristics. These changes occur as concentration alterations and composition modifications. Modifications to concentrations of plasma lipid have elicited attention in recent time. In a cross-sectional study in Anajas in Brazil, Neves *et al.* (2013) reported *Plasmodium* infection induced hypocholesterolemia. A systematic review and meta-analysis of data from major data bases (Medline/Pubmed, Embase, Cochranes Central Register of Controlled Trials, Web of Science, African Index Medicus, Biosis Previews, and Latin American and Caribbean Health Sciences Literature, LILACS) to determine malaria and other febrile diseases associated plasma lipid changes conducted by Visser *et al.* (2013) reported lower concentration of TC, HDL-C and LDL-C in malaria and other febrile diseases with malaria having a significant lowering effect compared to other febrile diseases. TG concentration increased though not significant in the malarious compared to control (Visser *et al.*, 2013). In Port Harcourt, Nigeria, Chikezie and Okpara (2013) reported a lower concentration of HDL-C and LDL-C in individuals with malaria compared to others without.The present study reported a lower concentration of TC, HDL-C and LDL-C in the pregnant women with malaria compared to their counterpart without.

From the present study, when comparisons were outlined based on the gravidity, parity, trimester of pregnancy, age and BMI of the pregnant women, significantly higher and non-significantly higher concentration of TC, LDL-C and HDL-C among the non-malaria associated pregnancy cases, except where age may be acting as a cofounder was observed. Variation in TG

concentration during pregnancy was not significantly dependent on malaria infection. Ayoola *et al.* (2012) made a similar observation from the comparison of the lipid profile at different trimesters of pregnancy. The TC, HDL-C and LDL-C were significantly lower in the malaria infected pregnant women compared to the uninfected at either trimester of pregnancy (Ayoola *et al.*, 2012).

Factors associated with pregnancy-associated malaria related changes in plasma lipid are diverse. Most protozoan parasites either completely lack or have an incomplete *de novo* lipid synthesis (Rub *et al.*, 2013). *Plasmodium* cannot synthesize fatty-acid and cholesterol *de novo* and consequently recruit these substances from the host cell (Labaied *et al.*, 2011). HDL-C is important for *P. falciparum* but within a specified range above which it becomes harmful to the parasite (Bansal *et al.*, 2005). Grellier *et al.* (1991) observed that while *P. falciparum* growth was complete *in vitro* in the presence HDL-C, it was incomplete in LDL-C medium and was stalled in very low density cholesterol (VLDL-C) medium. Grellier *et al.* (1990) showed that only in the presence of HDL-C was *P. falciparum* able to complete schizogony as well as normal erythrocytic re-invasion.

This view appeared to have been supported by the higher level of HDL-C in those with parasite density >20000/µL of RBC compared to the <20000/µL group in the age group 30 -39 (Figure 19). But the correlation result showed no significant correlation between parasite density and HDL-C. Thus, the nature of the rise in plasma HDL-C at parasite density >20000/µL in age group 30 ï 39 needs to be investigated. This is especially important as Labaied *et al.* (2011) did not observe a significant growth in liver stage *Plasmodium* parasite at increase concentration of HDL-C. The hepatocytic stage of *Plasmodium yoelli* and *Plasmodium berghei* have been shown to salvage cholesterol internalized by LDL-C from host cells (Labaied *et al.,* 2011).

Parasites may alter the human plasma environment by their secretory products. *P. falciparum* is known to export a number of proteins such as PfEMP-2, *P. falciparum* histidine rich protein (PfHRP)-1, and PfHRP-2 into host cell and extracellular milieu (El Tahir, 2003). Release from *P. falciparum*-infected intra-erythrocyte environment of extracellular vesicles laden with *falciparum* parasite molecules (Mantle *et al*., 2013) is yet another observation. These vesicles have also been demonstrated to be exuded by *Leishmania donovani* within a macrophage and were transported from the macrophage cytosol through the extracellular milieu into neighbouring

macrophages (Silverman *et al.*, 2010). Parasites may also release certain lipids (e.g. eicosanoids and ceramides) intracellularly and extracellularly to modulate selfishly immunologic activities of the host. The vast array of substances released by parasites may find their way into the plasma as foreign biochemical. They exact influence on disease outcome as they may modulate immunologic response in parasite favour, facilitate parasite establishment and proliferation, or define disease pathogenesis and the associated damages to the host.

The serum protein assayed in this study showed too much inconsistence as predictors of presence or absence of malaria in the pregnant women examined. Apart from the multigravidae that happen to fall under the multiparous category and the Ó30 BMI category and the 30 -39 age group, the A/G ratio appeared to maintain non-significantly ($P > 0.05$) lower value in the malaria infected than uninfected pregnant women for all the comparison between the malaria infected and uninfected women gravidity, parity, trimester of pregnancy, age and BMI. Amah *et al.* (2011) reported a lower A/G value in malaria infected patient compared to their uninfected control.

4.4 Conclusion

Pregnancy is associated with modifications to the plasma architecture of the gravidae. These modifications are in the form of changes to the levels of plasma biochemical such as total cholesterol, high density lipoprotein cholesterol, triglyceride, low density lipoprotein cholesterol, total protein, albumin and globulin. These alterations in plasma biochemical are directed at sustaining the foetus. Pregnancy-associated malaria may further alter the maternal plasma biochemistry interfering with the availability of substances to the foetus. High parasite burden and placental localization of *P. falciparum* is a major contributor to high pregnancy-associated malaria morbidities and mortality.

From this study, very significantly higher density of parasitaemia characterizes pregnancy-associated malaria in primigravid and nulliparous women. Non-usage of mosquito bed net unfortunately was more common among this group as well. Malaria in pregnancy indeed modifies plasma lipid and protein biochemistry. This is in addition to modifications that

characterize pregnancy progression and maternal age. The changes to maternal plasma lipid as a result of pregnancy and pregnancy-associated malaria are observable even in non-fasting state.

The relatively high prevalence of pregnancy-associated malaria and accompanying high parasitaemia among women attending antenatal clinics in Igbo-Eze North Local Government Area, Enugu State, Nigeria calls for urgent attention. Effort should be increased toward prompt malaria diagnosis and treatment. Diagnosis and treatment should be made mandatory and free, especially among the primigravid women. Increased awareness on mosquito bed net usage should accompany routine antenatal counselling. Distribution of treated mosquito bed net and introduction of intermittent preventive therapy will be of great help. There is need for more research to understand comprehensively pregnancy-associated malaria dynamics in order to reduce drastically, pregnancy-associated malaria morbidity and mortality in Igbo-Eze North Local Government Area.

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QUESTIONNAIRE

PLASMA LIPID, SERUM PROTEIN AND BODY MASS INDICES AMONG *FALCIPARUM*-MALARIA INFECTED WOMEN ATTENDING ANTENATAL CLINICS IGBO-EZE NORTH LOCAL GOVERNMENT AREA, ENUGU STATE, NIGERIA.

Please leave 'section A and C' only fill 'section B'. Only thick (√) where it applies to you.

SECTION A: For examiners user

1. Serial number_____
2. Date_____3. Time_____
4. Community_____ 5. Hospital_____

SECTION B: Thick (√) where it applies to you

I. Age

Less than 20 ☐ 20-29 ☐ 30-39 ☐ 40-49 ☐ 50-59 ☐

II. Gradidity

1st ☐ 2nd ☐ 3rd ☐ 4th ☐ 5th ☐ more than 5 ☐

III. Parity

1st ☐ 2nd ☐ 3rd ☐ 4th ☐ 5th ☐ more than 5 ☐

IV. Duration of Pregnancy

Less than 1 month ☐ 1month ☐ 2 months ☐ 3 months ☐

4 months ☐ 5 months ☐ 6 months ☐ 7 months ☐

8 months ☐ 9 months ☐ more than 9 months ☐

V. Any Strenous Exercise?

No ☐

Yes ☐ **When?** this morning ☐ Last night ☐ this afternoon ☐

VI. Eating?

No ☐ Yes ☐

VII. Any Health challenge?

No ☐

Yes ☐ thick (√) any: Diabetics ☐ liver disease ☐

High blood pressure ☐ Typhoid ☐ Others:_____

VIII. Take alcohol?

No ☐ Yes ☐

IX. Smoke?

No ☐ Yes ☐ ☐ ☐

X. Bednet Ownership and Usage

Do you have bednet? No ☐ Yes ☐

Do you use bednet? No ☐ Yes ☐

Section C: Personal Use (Do not fill)

XI. **Usage of Intermittent Preventive treatment with sulphadoxin-pyrimenthine (IPTp-SP)**

No ☐

Yes ☐ **How many times?** Once ☐ Twice ☐ Thrice ☐

More than thrice ☐

XII. Day of last malaria treatment?

Less than three days ago ☐ less than 7 weeks ago ☐ more than 3 weeks ☐

Other malaria preventive therapy?

No ☐

Yes ☐ **which?**_____

Antenatal visit

First ☐ Not first ☐

Anthropometric

Weight (kg)_____ Height (cm)_____