ASSESSMENT OF NUTRITIONAL STATUS AND ACUTE PHASE PROTEIN LEVELS OF PREGNANT WOMEN WITH *PLASMODIUM FALCIPARUM* ATTEND INGHEALTH CARE CENTERS IN SAMARU, KADUNA STATE

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OCTOBER, 2016

DECLARATION

I declare that the work in this dissertation entitled **"Assessment ofNutritional Status and AcutePhase Protein Levels of Pregnant Women with *plasmodium falciparum*Attending Health Care Centers in SamaruKaduna State",** has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for other degree or diploma at this or any other Institution.

Muhammad Sani ABUBAKAR .

MSc./Sci/04864/2011-2012 _____ _____

Signature Date

CERTIFICATION

I declare that the work in this dissertation entitled "**Assessment Of Nutritional Status And Acute Phase Protein Levels Of Pregnant Women With *Plasmodium Falciparum* Attending Health Care Centers in Samaru Kaduna State.**" by Muhammad Sani ABUBAKAR meets the regulations governing the award of the degree of Masters in Nutrition of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my children, Ahmad Muhammad Sani and Ibrahim Muhammad Sani.

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All praises and thanks be to Allah (SWT) the Almighty, Who gave little understanding to those who study metabolic aspect of life processes (Biochemist), in order to make the life of mankind more compatible.

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TABLE OF CONTENTS

Title Page ii

Declaration iii

Certification iv

Dedication v

Aknowlegement vi

Table of Contents viii

List of Tables xiv

List of Figures xv

List of Appendices xvi

List of Abbrevations/Symbols xvii

Abstract xix

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Background Information 1

1.2 Statement of Research Problem 4

1.3 Justification 4

1.4 Aim 5

1.4.1 Specific Objectives 5

1.5 Null Hypothesis **6**

CHAPTER TWO **7**

2.0 LITERATURE REVIEW **7**

2.1 Malaria in Pregnancy **7**

2.1.1 Micronutrient Deficiencies and Parasitic Infections 9

2.1.2 Call to Close Gaps in Prevention and Treatment to Defeat Malaria 12

2.2 Acute Phase Reaction and Acute Phase Proteins **13**

2.2.1 The Systemic Acute Phase Reaction 16

2.2.2 Cytokines and the Acute Phase Response 17

2.3 Acute Phase Proteins **20**

2.3.1 Negative Acute Phase Proteins 20

2.3.2 Positive Acute Phase Proteins 20

2.4 Function of Positive Acute Phase Proteins **23**

2.4.1 C - Reactive Protein (Crp) 23

2.4.2 Serum Amyloid A (Saa) 24

2.4.3 Haptoglobin (Hp) 25

2.4.4 Ceruloplasmin (Cp) 25

2.5 Acute Phase Proteins and Pathology **25**

2.6 Soluble Transferrin Receptors in Malaria **26**

2.7 Acute Phase Response in Animal Medicine **29**

2.8 Nutrients and their Role in Host Resistance to Infection 30

2.8.1 Iron (Fe) 31

2.8.1.2 Mechanisms for the Importance of Iron to the Immune System 33

2.9 Zinc (Zn) 33

2.9.1 Clinical and Experimental Evidence for the Essentiality of Zinc 34

2.9.2 Proposed Mechanisms for the Immune Essentiality of Zinc 35

CHAPTER THREE 37

3.0 MATERIALS AND METHODS 37

3.1 Materials 37

3.1.1 Reagents 37

3.1.2 Study Area 37

3.1.3 Sample Size 38

3.1.4 Study Population 38

3.1.4.1 Inclusion Criteria 39

3.1.4.2 Exclusion Criteria 39

3.1.5 Ethical Approval 39

3.2 Methods 39

3.2.1 Data Collection 39

3.2.2 Anthropometric Measurements 40

3.2.3 Laboratory Analysis 40

3.2.3.1 Sample Collection.....40

3.2.3.2 Haematological Parameters.....40

3.2.3.3 Assessment of Plasmodium Falciparum Infection.....40

3.2.4 Biochemical Procedure.....42

3.2.4.1 Determination of Zinc Using Water- Soluble Pyridylazo Dye (1984) 42

3.2.4.2 Determination of Copper Using Color 3, 5-Dibr- Paesa (1989) 43

3.2.4.3 Determination of Albumin Using Silverman Et Al., (1986).....44

3.2.4.4 Determination of Iron Using Ferro Zine Method, 1980......44

3.2.5 Quantitative Determinations of C-Reactive Proteins (Crp), Serum Amyloid A (Saa), Ferritin and Transferrin Using Enzyme–Immunosorbent Assay (Elisa) Test Kit. 46

3.2.5.1 C - Reactive Protein (CRP) Concentration Using 'Perfemed' Elisa Test Kit (Catalog No. 10603).....46

3.2.5.2 Ferritin Concentration using Ferritin Test System Product Code. 2825-300 (Monobind Inc. Lake Forest, Ca 92630, USA) 47

3.2.5.4 Human Serum Amyloid A (Saa) Concentration Using 'Perfemed' Elisa Test Kit (Catalog No. 10735) 50

CHAPTER FOUR 52

4.0 RESULTS.....52

4.1 Prevalence of *P. Falciparum* Infection in Pregnant Women by Healthcare Facility 52

4.2 Distribution of Pregnant Women Infected with *P. Falciparum* Based on Socio-Demographic Characteristics. 52

4.2.1 Use of Insecticides Treated Nets (ITNs) by Pregnant Women 53

4.2.2 Use of IPTp by the Pregnant Women and Frequency of Malaria Infection 53

4.3 Relationship between malaria parasite density, parity and gestation period..…...58

4.3.1 Birth Spacing Between Last and Current Pregnancy Among Pregnant Women 58

4.4 Indices of Iron Status Among Pregnant Women With *Plasmodium Falciparum* Infection 61

4.5 Nutritional Status of Pregnant Women with and Without *PlasmodiumFalciparum* Infection 61

4.6 Serum C-Reactive Protein and Malaria Parasite Density of *P. Falciparum* Infected Pregnant Women Attending PHCs in Samaru Zaria 64

4.6.1 Effect of Malaria Parasite Density (MPD) on Serum Amyloid A Of *P. Falciparum* Infected Pregnant Women Attending PHCs in Samaru Zaria. 64

4.6.2 Variability in Serum Amyloid A and C - Reactive Protein Based on Malaria Parasite Density 67

CHAPTER FIVE 69

5.0 DISCUSSIONS 69

CHAPTER SIX 77

6.0 CONCLUSION AND RECOMMENDATIONS 77

6.1 Conclusion 77

6.2 Recommendations.....77

REFERENCES 79

APPENDICES.....96

LIST OF TABLES

TABLE		PAGE
2.1	Major Positive and Negative Acute Phase Reactants in Mammals and Birds - - - - - - - - -	21
2.2	Summary of the Changes in Iron levels seen in Various Diseases Affecting Iron Status. - - - - - - - - -	28
4.1:	Prevalence of Malaria Parasite (*Plasmodium falciparum*) Among Pregnant Women by Health Care Facility - - - - - -	54
4.2:	Distribution of *P. falciparum* Infected Pregnant Women Based on Socio Demographic Characteristics - - - - - - -	55
4.3:	Relationship between malaria parasite density, parity and gestation -	59
4.4:	Relative Values of Indicators of Iron Status among Pregnant Women attending Health Care Facilities in Samaru, Zaria - - - -	62
4.5**:**	Relative Nutritional Status of Pregnant Women with and Without *Plasmodium falciparum* Infection... - - - - - -	63

LIST OF FIGURES

FIGURE PAGE

2.1 Cycle of adverse events throughout life associated with under nutrition and infectious diseases (ACC/SCN, 2000) - - - - 11

3.1 Experimental Design for P. falciparum screening parasite density and some Hematological parameters - - - - - - 41

4.1: Use of Insecticides Treated Nets (ITNs) by Pregnant Women attending Health Care Facilities in Samaru - - - - - - 56

4.2: The use of IPTp by the Pregnant Women and frequency of malaria infection 57

4.3: Birth Spacing between last and current among Pregnant Women - - 60

4.4: Scatter plot showing the relationship between C-reactive protein Concentration and Malaria parasite (P. falciparum) density in Infected Pregnant Women 65

4.5: Effect of Malaria Parasite Density (MPD) on Serum Amyloid A of *P. falciparum* Infected Pregnant Women attending PHCs in Samaru Zaria 66

4.6: Variability in Serum amyloid A and C - reactive protein as function of Malaria (P. falciparum) parasite density in Infected Pregnant Women - - 68

LIST OF APPENDICES

APPENDIX		PAGE
I	Assessment of Nutritional Status and Acute Phase Protein Levels among Pregnant Women with Malaria Questionnaire - - - -	97
II	C-reactive protein result Interpretation - - - - -	98
III	Human Serum Amyloid Interpretation - - - - -	101
IV	Human serum ferritin Interpretation - - - - - -	104
V	Human Transferrin Interpretation - - - - - -	107
VI	Ethical approval from University Medical Center ABU ZARIA - -	110
VII	Ethical approval from SabonGari LGA - - - - -	111
VIII	Informed consent - - - - - - - -	112

LIST OF ABBREVATIONS/SYMBOLS

ABBREVIATIONS	MEANING
ACC/SCN	Administrative Committee on Coordination/Sub Committee in Nutrition.
ACT	Artemisinin-based Combination Therapy
ACTH	Adrenocorticotrophic Hormone
AGP	α1-Acid Glycoprotein
ANOVA	Analysis of Variance
API	Acute Phase Index
APPs	Acute Phase Proteins
CRP	C-Reactive Protein
Hp	Haptoglobin
HRP2	Histidine Rich Protein 2
IL	Interleukin
IPTp	Intermittent Preventive Treatment
IRS	Indoor Residual Spraying
IRS	Indoor Residual Spray
ITNs	Insecticide-Treated Nets
ITNs	Insecticide Treated Mosquito Nets

IUGR	Intra Uterine Growth Retardation
LBW	Low Birth Weight
LLINs	Long Lasting Insecticide Nets
LPS	Lipopolysaccharide
NAPI	Nutritional and Acute Phase Index
RBP	Retinol binding protein
RDT	Rapid Diagnostic Test
SAA	Serum Amyloid A
sTfR	Soluble Transferrin Receptor
TNF	Tumour Necrosis Factor
TTR	Transthyretin

ABSTRACT

Malaria in pregnancy (MiP) is a serious public health problem which can be minimized by the use of Insecticides Treated Nets (ITNs) and Intermittent Preventive Treatment of malaria in pregnancy (IPTp). Inflammatory protein biomarker especially C-reactive protein and serum amyloid A can be used to detect MiP at subclinical level.A total of 547 pregnant women were screened from three Primary Health Centers (PHCs) Samaru, with 75*Plasmodium falciparum* positive. Malaria was diagnosed by both HRP2 (RDT) and Giemsa staining techniques, while socio-demographic data was obtained using semi-structured pretested questionnaires.Haemato-analyzer was used for Hb-concentration, leucocytes and platelets counts, while spectrophotometry was used for Iron, Zinc, Copper and Albumin estimations.ELISA technique was used for serum C-reactive protein, Amyloid A, Ferritin and Transferrin determinations. Results obtained showed 13.7% prevalence rate of *P. falciparum* infection.The peak age specific incidence was 15-19 years (17.5%), with mean parasite density of 5925. Those with no-formal education were affected mostly (17.4%) with parasite burden of 4419; while across occupation there was no difference in terms of infection rate and mean parasite density. Pregnant women were mostly predisposed to *P. falciparum* infection due to non-availability of ITNs, 41.3% of pregnant mothers had not use nets at all during pregnancy.Majority(68.3%) of the pregnant mothers complied with the use of IPTp, which may have led to reduced prevalence of *P. falciparum*infection in the study area. Incidence of *P. falciparum* infection varied significantly according to gestation period and parity, 19.9% for first trimester with mean parasite density of 7644 and primigravidae(19.0%) with mean parasite density of 9764 respectively. Patients with malaria and concomitant iron deficiency anemia (IDA) had mean sTfR level of 7.7 ±0.26 ng/ml (0.1- 5.0ng/ml), and Ferritin level of 385.8±56.6(22.5-278.6)pmol/L. Soluble

Transferrin in normal subjects and patients with*P*. *falciparum* malaria showed no significant difference ($p>0.05$). Statistically significant difference ($p < 0.001$) was observed in patients with malaria and concomitant IDA as compared to normal control group. This results showed that estimation of changes in some Acute Phase Proteins (CRP and SAA), despite the lack of diagnostic specificity may be useful to clinicians because such changes reflect the presence and intensity of inflammatory process (malaria infection), as well as trace element metabolism during malaria infection. The cohabitation of parasite manifestation and nutritional deficiency of trace elements creates damage in the health of infected population, due alteration in Zn/Cu ratio.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The scourge never ends'. This may be an apt description for one of the most prevalent infectious diseases-malaria. Globally, 3.3 billion people in 106 countries are at risk of malaria. In 2012, malaria caused an estimated 627,000 deaths, mostly among African children. Asia, Latin America, and to a lesser extent the Middle East and parts of Europe are also affected (WMD, 2014). Pregnant women and the under-fives form the bulk of its worst victims in endemic areas (Enato*et al.,* 2007).

Tackling malaria in pregnancy contributes to three of the Millennium Development Goals (MDGs), namely: goals 4 (To reduce child mortality), 5 (To improve maternal health) and 6 (To combat HIV/ AIDS, malaria and other diseases). Almost 30 million women are threatened by malaria in pregnancy (MiP) annually with about 10,000 maternal mortalities attributed to the disease each year and about 200,000 new born deaths annually (Mokuolu, 2011).

It is obvious that malaria in pregnancy causes tremendous strain on the already weakened health systems in endemic countries. The emergence of the human immunodeficiency virus (HIV) scourge has worsened the dimension of the already precarious condition. These all combined to make malaria infection a major public health problem in the tropics and subtropics(Cohen *et al*., 2005).

In the developing world, young women, pregnant women, their infants and children frequently experience a cycle where under-nutrition (macronutrient and micronutrient) and repeated infection lead to adverse consequences that can continue from one generation to

the next. Infants born prematurely or with low birth weight (LBW) are at increased risk of early death or at risk of poor growth and development in childhood and adolescence (Steketee*et al.,* 2001). The poor growth resulting in underweight and stunting leaves reproductive-age women at risk in their early pregnancies of delivering premature or LBW infants. In addition, the micronutrient deficiencies, particularly iron and folate deficiencies (which contribute to anemia), leave the young women at risk of anemia leading to inadequate oxygen-carrying capacity and risk in pregnancy of delivering premature or LBW infants (Steketee*et al.*,2006).

It was suggested that offspring of placental-infected multigravida women have the highest risk of *P. falciparum* during the first years of life compared to children of Primigravid placental non-infected women. *Plasmodium falciparum* is the only human malaria parasite with a clear and substantial adverse effect on pregnancy, nutrition during pregnancy and pregnancy outcome (Brabin, 1991). The first reaction of the body to immunological stress is the innate, non-specific response preceding specific immune reactions. The acute phase response (APR) is a prominent systemic reaction of the organism to local or systemic disturbances in its homeostasis caused by infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders (Gordon and Koy, 1985; Gruys*et al.,* 1999).

Intensive studies of the immune response to malaria parasites in human beings have provided a wealth of information about the cells and cytokines implicated in the pathophysiology of survival and fatal outcome in severe infections. This suggests that a crucial balance might exist during the inflammatory response to malaria infection and unbalanced response leads to severe disease. In mild malaria, inflammatory response might

be down regulated by anti-inflammatory cytokines, including interleukin 4, interleukin 10 and Transforming Growth Factor B (TGF-B) (Lucia and Salvatore, 2002).

The pathophysiological response to infection with *Plasmodium falciparum* is highly variable. It depends on several factors including previously acquired immunity and the production of cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), y-interferon (ɤ -IFN), and interleukin-6 (IL-6). Secretion of these mediators is thought to be responsible for many of the clinical features of malaria (Inigo and Manuel, 2002).

Although the stimulus to acute phase reactants production is not fully understood, macrophage derived cytokines including IL-6, TNF, and IL-1 are thought to be important (Gabay and Kushner, 1999).

Estimation of other changes in acute-phase proteins, despite the lack of diagnostic specificity, is useful to clinicians because such changes reflect the presence and intensity of an inflammatory process. Thus, measurements of plasma or serum C-reactive protein can be used to differentiate inflammatory from non-inflammatory conditions and are useful in managing the patient's disease, since the concentration often reflects the response to and the need for therapeutic intervention. In some diseases, such as rheumatoid arthritis, serial measurements of C-reactive protein are of prognostic value. Serum amyloid A concentrations usually parallel those of C-reactive protein, although some studies indicate that serum amyloid A is a more sensitive marker of inflammatory disease. At present, assays for serum amyloid A are not widely available (Gabay and Kushner, 1999).

C-reactive protein is a pentameric protein molecule which has been conserved over a long period of evolution. It binds to phosphorylcholine epitopes found in *Streptococcus pneumoniae* and many parasites, these binding results in the activation of .the classical

complement pathway. Increased concentrations of C-reactive protein have also been used to distinguish acute pyogenic from viral meningitis. Serum amyloid A is the precursor of amyloid A protein found in the amyloid fibrils present in secondary amyloid deposits. Both proteins increase in concentration in the serum up to 1000 times in response to inflammatory stimuli, whether infective or traumatic. Measurement of acute phase reactants like C-reactive protein or serum amyloid A has been used to monitor the response to antibacterial treatment (Gruys*et al.,* 2005).

1.2 Statement of Research Problem

Malaria infection (especially of *Plasmodium falciparum*) during pregnancy is a significant health problem with substantialrisk to the pregnant women, her fetus and new born child (WHO, 2013).

The use of cytokines for the diagnosis of malaria has been in practice for sometimes. But their short half-life in serum makes assay difficult to interpret for clinical purposes (Gabay and Kushner, 2009).

Acute phase proteins concentrations have been advocated as objective biochemical indices of disease activity in a number of different inflammatory processes. Though, it is not well documented whether parasitemia can mount a comprehensive acute phase protein response and if so, whether this is achieved by increasing acute phase protein synthesis due to the presence of malaria parasite (Yapi*et al.,* 2010).

1.3 Justification

An emerging view is that pathogenesis of malaria is a complex process in which a common outcome might be reached by different routes. This idea emphasizes the relevance of

diagnostic and prognostic parameters in predicting the specific risk associated with different clinical characteristics (Miller *et al.*, 2002).

This study was designed to test whether measurement of acute phase reactants instead of cytokine concentration would prove valuable in the diagnosis of malaria (especially of*Plasmodium falciparum*) in addition to standard laboratory methods, also whether sequential measurement would be valuable in assessing response to antimalarial treatment.

Clinical studies in large groups of patients with a variety of disorders confirmed the rapid production and exceptionally wide dynamic range of the serum amyloid A (SAA) response. It is as sensitive a marker for the acute phase as C- reactive protein (CRP). Therefore, determination of APPs can be used as fact in monitoring health of individual subjects especially when several acute phase variables are combined in an index (Gruys*et al.*, 2005).

1.4 Aim

To assess the nutritional status and some Acute Phase Proteins levels of pregnant women with*Plasmodium falciparum*attending three Primary Health Care centers, in Samaru Zaria.

1.4.1 Specific Objectives

i. To determine the prevalence rate of *P. falciparum* infection in pregnant women attending PHCs in Samaru Community.
ii. To document the socio-demographic characteristics of pregnant women with malaria (*Plasmodium falciparum*)and assess use of Intermittent Preventive Treatment for prevention of Malaria in Pregnancy (IPTp).
iii. Todetermine the relationship between malaria parasite density, parity and gestation period of pregnant mothers Infected with *Plasmodium falciparum*.

iv. To assesschanges in indices of Iron status (Fe, Hb, serum Ferritin/ Transferrin) during *Plasmodium falciparum* infection in pregnancy.

v. To determine relationship between the nutritional status of pregnant women with and without*Plasmodium falciparum* Infection.

vi. To determine the effect of malaria parasite burden on C-reactive protein and serum amyloid A among pregnant women with parasitemia.

1.5 Null Hypothesis

There's no relationship between Acute Phase Proteins (APP) production and malaria parasite density.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria in pregnancy

Malaria during pregnancy has been most widely evaluated in Africa south of the Sahara, with 90% global malaria burden (RBM, 2010). The burden of malaria infection during pregnancy is caused chiefly by *Plasmodium falciparum,* the most common species in Africa. The impact of the other three human malaria parasites (*Plasmodiumvivax, Plasmodiummalariae,* and *Plasmodiumovale)* is less clear. Every year at least 30 million pregnancies occur among women in malaria's areas of Africa, most of who reside in areas of relatively stable malaria transmission (WMD, 2014).

Although a few reports of adverse consequences of *Plasmodiumvivax* in pregnancy exist, *Plasmodium falciparum* is the only human malaria parasite that is more common in pregnant than in non-pregnant women and is the only human parasite with a clear and substantial adverse effect on pregnancy, nutrition during pregnancy and pregnancy outcome (Macgregor *et al*., 2003).

Erythrocytes infected with *Plasmodium falciparum* congregate in the maternal placental vascular space where the sinusoidal and low pressure blood flow, and possibly parasite adherence to endothelial cells (Beeson *et al*., 2001), allow parasites to sequester and replicate. An active immune response involving antibody production, cytokine release and a cellular response (principally a basophilic monocytes' macrophage response) is frequently observed in malaria-infected placentas (Richard and Steketee, 2003).

The infection and, possibly, aspects of the immune response contribute to poor pregnancy outcomes of prematurity and fetal intrauterine growth retardation (IUGR) (Menendez *et al*.,

2010). These adverse consequences appear to be mediated through several different pathways. The effect on prematurity is not entirely clear, but women with an active parasite infection and a fetus exposed to parasitized maternal erythrocytes (Steketee*et al*., 2006) may develop an immunologic response that contributes to stimulus of early onset of labor.

The effect of malaria on IUGR appears to be basic to the system of nutrient transport to the fetus. High-density or prolonged parasite infection in placental blood and the consequent cellular immune response may require substantial nutrients and thus leave fewer nutrients (glucose and oxygen) available for passage to the fetus. In addition, histopathology studies of malaria-infected placentas demonstrate thickening of cytotrophoblastic membranes, which may alter nutrient transport to the fetus (Ismail *et al*., 2008).

Although the details of these biological processes are difficult to study except when the placenta is delivered, the overwhelming evidence of many studies suggests a clear adverse effect of malaria on LBW and prematurity (Garner and Brabin, 1994). Malaria also clearly contributes to anemia throughout life and specifically during pregnancy. In a recent review of studies of *P. falciparum*–related anemia in pregnant women, (Guyatt and Snow, 2001) suggest that approximately 400,000 pregnant women develop moderate or severe anemia (hemoglobin <80 g/L or hematocrit <0.25) each year in sub-Saharan Africa as a result of malaria infection. Studies have shown that maternal anemia contributes independently to LBW through IUGR (Brabin and Piper, 2007) and to infant mortality.

Although the specific biological processes are not clearly delineated, the contribution of moderate and severe anemia to poor oxygen transport to the developing fetus is a likely mode of action for anemia's adverse effect on fetal growth. In addition, malaria-associated anemia in the mother likely has important consequences on her outcome whereby already

anemic women are at increased risk of severe consequences (e.g., hypotension, shock, death) even with a moderate ante- or postpartum hemorrhage (Verhoeff*et al*., 2010).

2.1.1Micronutrient deficiencies and parasitic infections

The consequences of parasitic infections on human nutrition and the consequences of under nutrition on parasitic infections and their outcomes are clearly intertwined. Recent reviews of micronutrient malnutrition and malarial anemia; malnutrition and parasitic helminthes infections and malnutrition, infection and immunity (Scrimshaw and Sangiovanni, 2007), suggest a strong link among protein-energy malnutrition, micronutrient deficiency, infection and bad outcomes. Although these interactions are not specific to pregnancy, the increased demands on micronutrients during pregnancy exacerbate the deficiency and its consequences (Stephenson *et al*., 2001).

In addition to their contribution to adverse pregnancy events and outcomes, micronutrient deficiencies may contribute to an increased risk of parasite infection or high-density infection. This increased risk has been suggested for iron, vitamin A and zinc (Baek*et al*., 1996). In developing countries where poverty is the norm and micronutrient deficiency (including vitamin A and zinc deficiency) and parasite infections are common, there remains a relatively limited set of clear treatment and supplementation recommendations that focus on iron, folate or treatment of malaria (WHO,2000) and intestinal helminthes (WHO, 2009).

Even for these clear recommendations, questions remain about optimal delivery strategies (Beard, 2000).Guidance for vitamin A and zinc supplementation in pregnancy exist but are somewhat more cautionary for vitamin A, with concern expressed on the need for

supervised delivery to address possible overdosing during pregnancy. For zinc the recommendations are more focused on specific populations of women at particular risk of deficiency (King, 2010).

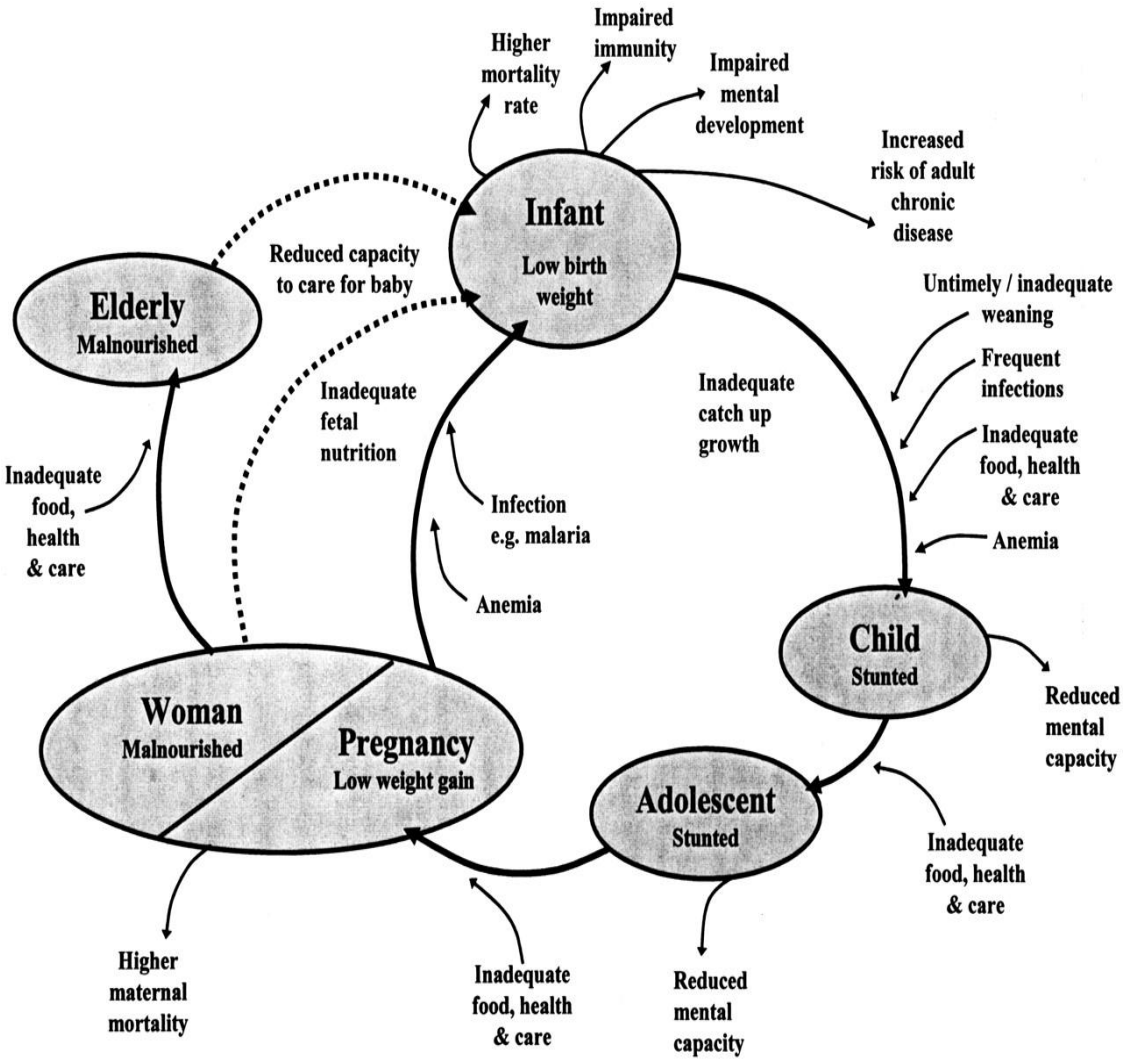


Figure 2.1:Cycle of adverse events throughout life associated with under nutrition and infectious diseases. Infections (e.g., malaria) increase nutrient requirements and contribute during pregnancy to anemia and low birth weight during childhood and child stunting (World Health Report, 2013)

2.1.2 Call to close gaps in prevention and treatment to defeat malaria

Increased political commitment and greater funding have averted more than 4 million malaria deaths since 2001, and 55 of the 97 countries and territories with ongoing malaria transmission are on track to meet the current World Health Assembly target of reducing malaria incidence by 75% between 2000 and 2015 (WMD, 2015).

An estimated 15 million of the 35 million pregnant women in SSA did not receive a single dose of IPTp in 2013 (World Malaria Report 2014).The intermittent preventive treatment against malaria in pregnancy (IPTp) is a highly cost-effective intervention with the potential to save many maternal and neonatal lives. However, IPTp coverage remains low in sub-Saharan Africa where immediate action is needed for dramatic scale up. While some obstacles to IPTp uptake relate to large health-systems issues, many barriers are common across countries and could be overcome with relative ease and speed (WHO, 2012).

In accordance with the Millennium Development Goals, the Abuja Declaration on Roll Back Malaria in Africa, the goals contained in the outcome document of the UN Special Session on Children: "A World Fit for Children," and updated targets contained in the Roll Back Malaria Strategic Plan 2005-2015, UNICEF aims to help ensure that(RBM, 2010):

By 2010, particularly in the lowest two economic quintiles:

(i) Eighty (80%)per cent of people at risk from malaria are protected, through the use of locally appropriate vector control methods such as insecticide-treated nets (ITNs), and, where appropriate, indoor residual spraying (IRS) and, in some settings, other environmental and biological measures;

(ii) Eighty (80%)per cent of malaria patients are diagnosed and treated with effective antimalarial medicines, e.g. artemisinin-based combination therapy (ACT) within one day of the onset of illness;

(iii) In areas where transmission is stable, 80 per cent of pregnant women receive intermittent preventive treatment (IPTp);

(iv) Malaria burden is reduced by 50 per cent compared with 2000.

And by 2015:

(i) Malaria morbidity and mortality are reduced by 75 per cent in comparison with 2005, not only by national aggregate but particularly among the poorest groups across all affected countries;

(ii) Malaria-related Millennium Development Goals are achieved, not only by national aggregate but also among the poorest groups, across all affected countries;

(iii) Universal and equitable coverage with effective intervention (RBM, 2010).

2.2 Acute phase reaction and acute phase proteins

A review of the systemic acute phase reaction with major cytokines involved, and the hepatic metabolic changes, negative and positive acute phase proteins (APPs) with function and associated pathology is given. It appears that APPs represent appropriate analytes for assessment of animal health. Whereas they represent non-specific markers as biological effect reactants, they can be used for assessing nutritional deficits and reactive processes, especially when positive and negative acute phase variables are combined in an index. When such acute phase index is applied to separate healthy animals from animals with some

disease, much better results are obtained than with single analytes and statistically acceptable results for culling individual animals may be reached (Gruys*et al*., 2005.).

Unfortunately at present no cheap, comprehensive and easy to use system is available for assessing various acute phase proteins in serum or blood samples at the same time. Protein microarray or fluid phase microchip technology may satisfy this need; and permit simultaneous analysis of numerous analytes in the same small volume sample and enable integration of information derived from systemic reactivity and nutrition with disease specific variables (Abdalla*et al*., 2004).

The cytokines activate receptors on different target cells leading to a systemic reaction resulting in activation of the hypothalamic-pituitary-adrenal axis, reduction of growth hormone secretion (Gruys*et al*., 1999) and a number of physical changes clinically characterized by fever, anorexia, negative nitrogen balance and catabolism of muscle cells (Dinarello, 1989). Furthermore a series of changes can be measured in the laboratory: such as:

i. A decrease of blood plasma low and high density lipoprotein-bound cholesterol and leukocyte numbers in blood,
ii. Increased values of adrenocorticotrophic hormone (ACTH) and glucocorticoids,
iii. Activation of the complement system and blood coagulation system,
iv. Decreased serum levels of calcium, zinc, iron, vitamin A and of α-tocopherol, and
v. A change in concentration of several plasma proteins, the acute phase proteins (APPs) (Dinarello, 1989; Gruys *etal*., 1999) largely due to a changed hepatic metabolism. When the receptor triggering has repeated pulses, the acute phase response can become chronic.

Within a few hours after infection the pattern of protein synthesis by the liver is drastically altered resulting in an increase of some blood proteins, the positive APPs (Kushner *et al*., 1981; Blackburn, 1994; Ingenbleek and Young, 1994). Hepatic mRNA up regulation of those APPs is associated with a decrease in synthesis of normal blood proteins, like transthyretin (TTR, formerly called prealbumin), retinol binding protein (RBP): cortisol binding globulin, transferrin and albumin, which represent the negative APPs. The positive APPs are mainly the proteins, C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) which are released by the hepatocytes after cytokine stimulation (Heinrich *et al*., 1998).

Infection burdens often are associated with growth failure. The high prevalence of infections among children living in poor areas of developing countries impairs linear growth in these populations (Stephensen, 1999). Acute, invasive infections which provoke systemic response such as dysentery and those causing pneumonia, and chronic infections which affect the host over a sustained period (e.g., enteric helminthes infections), have substantial effect on linear growth.

This occurs because the infections may decrease food intake, impair nutrient absorption, cause direct nutrient losses, increase metabolic requirements and catabolic loss of nutrients and may impair transport of nutrients to target tissues.

The acute phase response with its changes in blood plasma composition is thought to be beneficial to the organism by preventing microbial growth and helping to restore homeostasis. Some APPs opsonize microorganisms and activate complement, others scavenge cellular remnants and free radicals, or neutralize proteolytic enzymes.

2.2.1 The systemic acute phase reaction

Increases or decreases in APPs serum concentration are observed after 2-6 days of disease. Increases of concentration predominate, with wide range, one-fold to two-fold in some complement proteins, five-fold to ten-fold in coagulation factors and protease inhibitors, hundred-fold to thousand-fold in CRP and SAA. The maximal level could be seen after 8-10 days and it is maintained during all evolution of disease (Tirziu*et al*., 2009).

Local inflammation is the major reaction of the body upon tissue injury caused by infection. Infection, however, may occur without inflammation e.g., in immune-compromised individuals. Inflammation may also develop due to non-infectious causes. Any tissue damage during these processes leads to release of pro-inflammatory cytokines (Van Miert, 1995). These cytokines, nitric oxide and glucocorticoids trigger and modulate the systemic acute phase reaction and the hepatic acute phase protein response (Gruys*et al*., 1994; Van Miert, 1995). Protein-malnutrition and long-term starvation or anorexia, however, can reduce or abrogate a full positive acute phase protein reaction, while reducing the negative acute phase reactants by the starvation process itself. The same holds for hepatic impairment.

Bacterial infections usually lead to a strong systemic acute phase response (Alsemgeest*et al*., 1994), due to the strong reaction of the mononuclear-phagocytic system's cells. TNF-α and IL-1β are induced in response to endotoxin (Dinarello, 1983; Werling*et al*., 1996). In viral infections, generally the APR is milder (Nakayama *et al*., 1993; Alsemgeest*et al*, 1994). The main cytokines then released by infected cells are primarily interferon's (IFNs), especially IFNγ from mononuclear inflammatory cells, although TNF-α and IL-1β from

tissue cells may be involved as well.When severe cellular destruction is present, a full APR can be observed (Van Reeth*et al*., 1998).

2.2.2Cytokines and the acute phase response

At least 15 different low molecular weight peptide mediators are known to be secreted by activated leukocytes (interleukins') and other cells. They are collectively termed cytokines and are involved in triggering the acute phase response.

Three main groups of cytokines corresponding to effect pathways can be distinguished (Van Miert, 1995):

(i) Cytokines that primarily act as positive or negative growth factors for a variety of cells (IL-2, IL-3, IL-4, IL-7, IL-10, IL-1, IL-12 and granulocyte-macrophage colony stimulating factor),

(ii) Cytokines with pro-inflammatory properties (TNF-α/β, IL-1α/β, IL-6, IFN-α/γ, IL-8, and macrophage inhibitory protein-1), and

(iii) Factors with anti-inflammatory activity (IL-1 receptor antagonists, soluble IL-1 receptors, TNF-α binding protein and IL-1 binding protein).

The pro-inflammatory cytokines (those of the second group) are responsible for induction of the mentioned fever and muscle catabolism, and they activate white blood cell precursors in the bone marrow, growth of inflammatory tissue fibroblasts and macrophages (Dinarello, 1989; Chang*et al*., 1991; Van Miert, 1995). They are responsible for a broad spectrum of synergistic or antagonistic effects that influence the specific immune response of the stressed organism against foreign antigens and invading microorganisms (Van Miert, 1995). TNF-α, IL-1β and IFNγ are crucial for theinduction of other cytokines (IL-6 and IL-8) and

agents such as platelet activating factor, prostaglandins, leukotriene and nitric oxide (Van Miert, 1995).

In the hepatic APR, TNF-α, IL-1 and IL-6 play a key role (Heinrich *et al*., 1998; Ingenbleek and Young, 1994; Chang*et al*., 1991). They activate hepatocytic receptors, and synthesis of varying APPs starts. IL-6 is the major mediator for the hepatocytic secretion of most of the APPs (Heinrich *et al*., 1998; Lee and Vilcek, 1989; Chang*et al*., 1991). Furthermore, TNF-α causes muscle catabolism that is also mediated by glucocorticoids, as well as glucagon-induced hyperglycemia and amino acid uptake by the liver. IL-1 stimulates an increase in whole body amino-acid flux, and activation of the pituitary-adrenal system. It has been shown that Kupffer cells play an intermediate role (Knolle*et al*., 1995). After stimulation by the pro-inflammatory cytokines the Kupffer cells form IL-6 and present it to the hepatocytes. The IL-6 depresses mononuclear phagocytic production of IL-1 and TNF-α (Schindler *et al.*, 1990) thus mitigating the whole cascade reaction. Down-regulation of the hepatocytic APR is achieved by rapid hepatic removal of circulating cytokines (Heinrich *et al*., 1998), release of IL-10 by the Kupffer cells which results in suppression of the local IL-6 production (Knolle*et al*., 1995) and by gene suppression pathways coactivated on receptor binding (Heinrich *et al.*, 1998). Receptors for the proinflammatory cytokines may induce a janus-kinase effect resulting in activation of the APP formation pathway as well as several receptor inhibiting pathways (Heinrich *et al*., 1998). Moreover, parts of the hepatic APR are suppressed by IL-1 and IL-4 (Loyer*et al*., 1993) and some acute phase proteins can modulate monocyte cytokine production (Pue*et al*., 1996).

The glucocorticosteroids have a double function: (1) glucocorticosteroid dependent (Heinrich *et al*., 1998) hepatic stimulation of hepatocytes by IL-6; (2) the steroids down-

regulate cytokine production by monocytes and macrophages (Baybutt and Holsboer, 1990). The APR observed is the resultant of all these complex interactions. It is important to realize that the APP-response-inducing cytokines represent small molecules with very short half-life. Therefore, cytokines are not very useful for most general diagnostic purposes, in contrast to the APPs (Blackburn, 1994; Gruys*et al*., 1994; 1999).

Sickness behavior with decreased appetite or anorexia is mediated by the pro-inflammatory cytokines. The cytokines induce formation of prostaglandins and the prostaglandin-dependent induction of fever (Johnson and Borrell, 1994;Van Miert, 1995). Furthermore, the immunological stress induces adrenal gland medullary hormone release with catecholamines causing re-distribution of the blood flow to brain and muscles instead of the splanchnic system. Intestinal villus atrophy and reduced enteric absorption may develop and result in diarrhea (Kraft *et al.*, 1992; Nabuurs, 1995). The changed metabolism results in negative energy balance and growth retardation is ameliorated.

During the acute phase response, plasma viscosity increases as a result of the total changes in total blood protein concentration, among which an increase of fibrinogen which influences the erythrocyte sedimentation rate (ESR) used in many western hospitals as non-specific marker for disease activity (Magnus *et al*., 1994). Because fibrinogen is a slow reacting positive acute phase reactant with a possible delay of some days after infection, the ESR increases and then reflects the activity of the acute phase response. The ESR was found to be useful for monitoring pigs with abscesses (Odink*et al*., 1990). In cows fibrinogen and the ESR are not reliable indicators of activity of the acute phase response.

2.3Acute Phase Proteins

2.3.1Negative acute phase proteins

In addition to the decrease in serum zinc, iron and albumin, a decrease of transferrin, cortisol-binding globulin, transthyretin (TTR) and retinol-binding protein (retinol = vitamin A) have also being described (Ingenbleek and Young, 1994). Their decrease indicates a temporarily increased availability of free hormones bound to those proteins. The negative acute phase proteins are therefore described by some authors as ' acute booster reactants ' (Ingenbleek and Young, 1994).

In malnutrition and chronic infections the response of positive acute phase variables may be less evident (Morlese*et al*., 1998). Changes in blood protein profiles partly depend on starvation and muscle catabolism (Reeds *et al*., 1994). In chronic infestation and inflammatory states of children and during pregnancy in developing countries in addition to malnutrition, vitamin A deficiency is worsened (Stephenson*et al*, 2001). The latter has a well-known negative feedback effect on immunity (Baeten*et al*., 2004; Stephenson*et al*, 2001).

2.3.2 Positive acute phase proteins

Although species-differences exist for separate proteins and especially are known between mammals and birds (Table1), the positive APPs of man and domestic animals (Kushner *et al*., 1981; Lannergard*et al*., 2003) can generally be listed in three major groups: (1) with an increase of about 50%: ceruloplasmin and complement factor-3 (C3), (2) with an increase of two-three fold: haptoglobin, fibrinogen, α-globulins with antiprotease-activity and lipopolysaccharide binding protein, and (3) with a rapid increase of up to 5-fold to 1000-fold: CRP and SAA.

Table 2.1 Major Positive and Negative Acute Phase Reactants in Mammals and Bird. Source; (Gruys*et al*, 2005)

Mammals	Birds
Positive reactants	
TNF-α, IL-1, IL-6, cortisol	TNF-α, IL-1, IL-6, cortisol
SAA, CRP, Hp, AGP, etc.	SAA, CRP, hemopexin, AGP, etc.
Fibrinogen, Ceruloplasmin	Fibrinogen, Transferrin, Ceruloplasmin
Cu	Cu, Ca
Negative reactants	
TTR, RBP	Hp
Albumin, Transferrin	Albumin
Fe, Zn, Ca	Unbound serum iron, Zn

TNF: Tumour necrosis factor; IL: Interleukin; SAA: Serum amyloid A; CRP: C-reactive protein; Hp: Haptoglobin; AGP: α1-acid glycoprotein; Cu: Copper; Ca: Calcium; TTR: Transthyretin; RBP: Retinol binding protein; Fe: Iron; Zn: Zinc

Some of the APPs are fetal proteins normally not found in large quantities in sera of adult subjects, e.g., α-macrofoetoprotein in the rat (Van Gool*et al*., 1984) and α1-acid glycoprotein (AGP) in most animal species.

Positive acute phase proteins are formed during the acute phase response associated with anorexia and changed metabolism. This indicates that rather than the role of protein absorption in the digestive tract, muscle protein functions as major storage for the amino acids required for AP synthesis. Since the amino acid composition of the APPs differs from that of muscle protein, the demands for phenylalanine, tryptophan and tyrosine together necessitate the mobilization of an amount of muscle protein that is considerably in exceeding (thrice) the quantity of the APP synthesized (Reeds *et al*., 1994). To minimalize muscular catabolism, for hospitalized acute phase patient's protein diets have been recommended (Alexander *et al*., 1980) which are now beginning to be given to pigs and chickens as well.

It is important to realize that physiologically, APPs may react at pregnancy (Eckersall*et al*., 1993) and parturition in the adult animal (Uchida *et al*., 1994:Sordillo*et al*., 1995:Koets*et al*., 1998) like man (de Villiers *et al*., 1990). Distinct positive APPs from some species do not react in the same way in other species; serum amyloid P-component (SAP) is an APP in the mouse, but not in man, and CRP reacts as APP in several monogastric species, but not very well in small ruminants (Gruys*et al*., 1994). Transferrin, which is a negative APP of most mammalian species, reacts as positive APP in chicken (Hallquist and Klasing, 1994).

The plasma concentration of APPs measured is the resultant of production and catabolism. SAA and CRP, become measurable within 4~5h after a single inflammatory stimulus. After a single stimulus the levels of these proteins remain elevated for a minimum of 24 h and decrease after about 48 h. Permanent infusion of endotoxin in cows causes plasma SA

quantities to remain on a plateau (Werling*et al.*, 1996). During permanent stimulation (chronic infection) positive acute phase protein levels remain elevated in comparison to normal values, and can be used for diagnostic purposes.

2.4Function of positive acute phase proteins

The function of most APPs has not been totally elucidated. The positive APPs are regarded as having general functions in opsonization and trapping of micro-organisms and their products, in activating complement, in binding cellular remnants like nuclear fractions, in neutralizing enzymes, scavenging free hemoglobin and radicals, and in modulating the ghost's immune response(Gabay and Kushner, 1999).

2.4.1 C - reactive protein (CRP)

It was discovered due to its binding to the C-polysaccharide of pneumococci. It binds directly to several microorganisms, degenerating cells and cell remnants, and activates complement by the classical C1q pathway and acts as opsonic.Binding of CRP to thebacteria, fungi and parasites membranes areCa^{2+} dependent, being associatedwith the classical activation pathway of the complement, resulting C3b formation.After interaction with various ligands, CRP exerts other functions, similar toantibodies - like agglutination – but without specificity. CRP reacts also like opsonic.

It amplified chemo taxis mechanisms and macrophages and neutrophils phagocytosis. CRP binds damaged cells nuclear chromatin, participating in degradation of their nuclear components. CRP also binds some endogenous compounds, like membrane phospholipids.

Similar to other APPs, CRP is synthesized by hepatocytes, synthesis amplified in acute infections, inflammations, neoplastic and autoimmune diseases, and diseases with immune

complexes. In these pathological conditions, CRP serum concentration increases rapidly, being obvious in a few hours after infection (de Villiers *et al*., 1990).

Conclusively, CRP has nonspecific functions, independent to immune system, in early response and defense mechanisms against infectious diseasesand other pathological conditions.

2.4.2Serum amyloid A (SAA)

Serum amyloid A (SAA) has various functions, including decrease of IL-1 and TNF-α, inhibition of thrombocytes aggregation, and inhibition of oxidative reaction in neutrophils. Higher plasma concentration of SAA determined amyloidosis, disease characterized by deposition of this APP in various tissues, in fibrils that interfere with normal functions of the organs (e.g. myocardial contraction and glomerular filtration).

SAA (Gruys*et al*., 1994; Husby*et al*., 1994; Nakayama *et al*., 1993) is an Apo lipoprotein of high-density lipoprotein (apo-SAA). As acute phase protein it is thought to influence high-density lipoprotein-cholesterol transport. In tissues it attracts inflammatory cells and inhibits the respiratory burst of leukocytes and modulates the immune response (Gruys*et al*., 1994). It is described to bind lipopolysaccharide, comparable to lipopolysaccharide binding protein (LBP) (Schroder*et al*., 2001). Several iso-types of SAAare found; types 1 and 2 represent positive APPs. In the bovine, also a negative protein cross-reacting with anti-SAA serum has been described (Yamamoto *et al*., 1998).

Besides the acute phase SAAs, constitutive variants are described (Husby*et al.*, 1994). The mammary gland is a well-known source of a Sea variant (Eckersall*et al*., 1993; Larson *et al.*, 2005) occurring in colostrum and in mastitis milk, that should have beneficial functions for the gut mucosa of the offspring.

2.4.3 Haptoglobin (Hp)

Haptoglobin (Hp) strongly binds haemoglobin, has anti-inflammatory capabilities and binds to CD11b/CD18 integrines (El Ghmatiand Van, 2008) representing major receptors on the cell membranes of leukocytes. Although representing a positive acute phase protein, its quantity may decrease on massive erythrolysis (Smith and Roberts, 1994), and when blood is hemolytic, determination by haemoglobin binding assays may give unreliable results.

2.4.4 Ceruloplasmin (Cp)

Ceruloplasmin (Cp)(Cooper and Ward, 1979) contains copper, has histaminase-and peroxidase-activity, and scavenges Fe^{2+} and free radicals, while α2-macroglobulin (α2MG) binds proteolytic enzymes (Alsemgeest*et al*, 1993). The function of fibrinogen is clot-formation and C3 has complement function; α1-acid glycoprotein (α1AGP), formerly called orosomucoid (Cooper and Ward, 1979) and which has been found not to react as a major acute phase protein in most domestic animal species except the cat, is reported to influence T-cell function and to bind steroids such as progesterone. The functions of α1-proteinase inhibitor which is also called α1-antitrypsin, a serine protease inhibitor or serpin, and α1-antichymotrypsin are inhibitors of leukocyte and lysosomalproteolytic enzymes (Cooper and Ward, 1979).

2.5Acute phase proteins and pathology

Some disease states are associated with, or are causally related to APPs. The pathogenic role of fibrin in thrombosis is well known. Similarly, CRP-mediated complement activation has a key role in some forms of tissue alteration such as cardiac infarction. Elevated serum values are known to be associated with increased risk of human atherosclerosis. Many studies had been conducted on the relationship between SAA and deposition of reactive (a)

amyloid (Gruys and Snel, 1994) in patients with chronic arthritis, tuberculosis or Familial Mediterranean fever.

A-amyloid is the most frequently found type of amyloid in animal species like horse, cow, dog, cat, acaridae and domestic fowl (Gruys and Toussaint, 1994). The causal relationship between the acute phase protein, SAA, and the extracellular deposition of amyloid fibrils has been proven (Gruys*et al*., 2005; Tape *et al*., 1988). In some species (mouse, mink and horse) a special amyloidogenic SA-isotype has been recognized. The mechanism of amyloid formation from the acute phase protein, however, has not totally been elucidated. Sustained high plasma levels of SA, amyloid enhancing factor, impairment of SA-degrading proteases, apolipoprotein-E4 (Gruys*et al*., 2005), and many other factors such as proteoglycans (Magnus *et al*., 1994), have been implicated.

2.6 Soluble Transferrin Receptors in Malaria

Soluble transferrin receptors (sTfR) level has been suggested to be a sensitive indicator of iron status in adults. Human transferrin receptor (TfR) is transmembrane dimeric glycoprotein. It is composed of two identical 95 kDa sub-units linked by disulphide bonds. sTfR is a truncated form of the intact transferrin receptor and circulating as a complex of transferrin and its receptors (Phir*et al.*, 2009). Their molecular mass is 85 kDa. About 80% of the circulating sTfR originate from erythroid precursors.

Unlike other laboratory tests of iron status (serum iron, TIBC, % transferrin saturation and serum ferritin), sTfR levels are not affected by infections and inflammation in the absence of iron deficiency (Ferguson *et al*., 1992)sTfR levels constitute a reliable test for the diagnosis of iron deficiency in patients with concurrent malarial infection.This test, therefore, has an edge over the conventional serum iron profile which fails to give the

expected results in this scenario. There are only a few reports on the application of sTfR levels in the diagnosis of iron deficiency and concomitant malaria in patients living in endemic areas (Williams *et al*., 1999).

The results of transferrin tests, TIBC, or UIBC are usually evaluated in conjunction with other iron tests. A summary of the changes in iron tests seen in various diseases of iron status is shown in the table below.

A high TIBC, UIBC, or transferrin usually indicates iron deficiency, but they are also increased in pregnancy and with the use of oral contraceptives.

A low TIBC, UIBC, or transferrin may occur if someone has:

- Hemochromatosis
- Certain types of anemia due to accumulated iron
- Malnutrition
- Inflammation
- Liver disease
- Nephritic syndrome, akidney disease that causes a loss of protein in urine.

Transferrin saturation is decreased with iron deficiency and increased when excess amounts of iron are present, as in iron overload or poisoning (Gersten, 2013).

Table 2.2: Summary of the Changes in Iron levels seen in Various Diseases Affecting IronStatus.

Disease	Iron	TIBC/ Transferrin	UIBC	%Transferrin Saturation	Ferritin
Iron Deficiency	Low	High	High	Low	Low
Hemochromatosis	High	Low	Low	High	High
Chronic Illness	Low	Low	Low/Normal	Low	Normal/ High
Hemolytic Anemia	High	Normal/Low	Low/Normal	High	High
Sideroblastic Anemia	Normal/ High	Normal/Low	Low/Normal	High	High
Iron Poisoning	High	Normal	Low	High	Normal

2.7Acute Phase Response in Animal Medicine

CRP, SAA, Hp and some other APPs, have been described as useful for assessing health in human patients (Blackburn, 1994; Ferard*et al*., 2002) and in various domestic animals (Gruys*et al*., 1994).

They are more sensitive than ESR, which is used in most western human hospitals. The significance of APP as non-specific variables for monitoring inflammatory activity has been adopted in veterinary clinical chemistry. Cytokines and the acute phase reaction elicited have been published for ruminants, horse, pig and several other species (Gruys*et al*., 1994). APPs are used with conditions varying from cows with mastitis (Eckersall*et al*., 2001), to cattle with tropical theileriosis (Glass *et al*., 2003), and horses with influenza (Hulten*et al*., 1999).

When APPs are used to assess unhealthy animals versus healthy ones, values of single reactants are often not sensitive enough to detect a special subject in a population of livestock. However, the acute phase signal/starvation situation obtained for an individual animal can be enhanced when the values of positive APPs (rapid and slow) are combined with those of rapid and slow negative APPs in an index (acute phase index (API) (Toussaint *et al*., 1995) or nutritional and acute phase indicator, NAPI) (Gruys*et al*, 2005).

$$\text{NAPI} = \frac{\text{(Value of a rapid positive APP} \times \text{Value of a slow positive APP)}}{\text{(Value of rapid negative APP} \times \text{Value of a slow negative APP)}}.$$

The index has been used as prognostic inflammatory and nutritional index (PINI) for human patients (Bonnefoy, *et al*., 1998) and as acute phase index (API) for cattle (Toussaint *et al*., 1995). Such index enhances sensitivity and specificity remarkably in comparison to single

APPs in the detection of unhealthy subjects among populations of normal animals, as was shown for cattle and finishing pigs at slaughter (Toussaint*et al*., 2000) and was favoured by findings in experimental pigs with Streptococcus species infection. In human patients a simple quotientof the values of CRP/TTR already proved its usefulness in monitoring bone fracture patients (Ferard*et al*., 2002).

2.8Nutrients and their role in host resistance to infection

Almost all nutrients in the diet play a crucial role in maintaining an "optimal" immune response, such that deficient and excessive intakes can have negative consequences on immune status and susceptibility to a variety of pathogens. Iron and vitamin A deficiencies and protein-energy malnutrition are highly prevalent worldwide and are important to the public health in terms of immune-competence. There are also nutrients (i.e., glutamine, arginine, fatty acids, and vitamin E) that provide additional benefits to immune-compromised persons or patients who suffer from various infections. The remarkable advances in immunology of recent decades have provided insights into the mechanisms responsible for the effects of various nutrients in the diet on specific functions in immune cells. In this review, we will present evidence and proposed mechanisms for the importance of a small group of nutrients that have been demonstrated to affect host resistance to infection. An inadequate status of some of these nutrients occurs in many populations in the world (i.e., vitamin A, iron, and zinc) where infectious disease is a major health concern. We will also review nutrients that may specifically modulate host defense to infectious pathogens (long-chain polyunsaturated n-3 fatty acids, vitamin E, vitamin C, selenium, and nucleotides). A detailed review of the effect of long-chain polyunsaturated n-3 fatty acids on host defense is provided as an example of how the disciplines of nutrition and

immunology have been combined to identify key mechanisms and propose nutrient-directed management of immune-related syndromes (Catherine*et al,* 2012).

2.8.1Iron (Fe)

Iron deficiency is estimated to affect 20–50% of the world's population, making it the most widespread nutritional deficiency in industrialized and developing countries. The physiological effects of iron deficiency are manifest in a large number of tissues including those of the immune system. The interrelationship between iron deficiency, toxicity, and immunity is complex, and many excellent reviews have been published (Beard, 2001).

2.8.1.1 Iron and immune function

It is well-documented that iron regulates the function of T lymphocytes, and in most studies (in vivo and in vitro), a deficiency results in impaired cell-mediated immunity. Iron deficiency may also delay the development of cell-mediated immunity. Immune cells appear to differ from one another in their synthesis and use of iron-binding proteins and in the amount of iron they take up and store; this suggests that there would be differential effects of iron status on various immune functions (Brock and Mulero, 2000). Numerous studies describe normal T-lymphocyte-proliferative responses to mitogens and limited effect on other immune functions in humans and animals with mild-to-moderate iron deficiency (Beard, 2001). This suggests that the immune response may not be impaired by alterations in iron availability to the same extent as are other organs. Indeed, lymphocytes meet an increased iron requirement during proliferation or other conditions by increasing the synthesis and expression of surface transferrin receptors (Seligman *et al*., 1992). Humoral immunity may be less affected by iron deficiency than cellular immunity, because antibody

production in response to immunization with most antigens is preserved in animals and humans with poor iron status (Oppenheimer, 2001).

Macrophage phagocytosis is generally unaffected by iron deficiency, but bactericidal activity of these cells is attenuated. It has been proposed that the immune surveillance role of macrophages may be mediated in part by modulation of iron status in cells (Weinberg, 1992). Unlike other cells, macrophages acquire iron for metabolic use via phagocytosis of affected erythrocytes, which is subsequently released into the circulation, where it is bound to transferrin and available to other cells. When activated (i.e., during inflammation, possibly signaled by IL-1 and IFN-γ), macrophages increase their uptake of iron and bind it in the cells (via increased transferrin receptors and ferritin synthesis (Brock and Mulero, 2000).

This sequestration of iron in macrophages has been proposed to be beneficial during the early, acute stages of infection with pathogens, because it would limit availability to microorganisms (particularly intracellular microorganisms); however, it would also limit availability to other immune cells, and this would impair host resistance (Oppenheimer, 2001).

Many of the immune abnormalities associated with iron deficiency appear to be reversible with iron repletion, but this has been difficult to demonstrate in clinical and observational studies. Experimental and clinical data suggest that there is an increased risk of infection during iron deficiency, although a few studies indicate otherwise (Oppenheimer, 2001).

Interpretation of many of the human studies is confounded by the existence of multiple nutrient deficiencies and uncontrollable environmental factors associated with poverty.

Experimental studies in laboratory animals uniformly show reversible deleterious effects of iron deficiency on most measures of functional immunity (Beard, 2001).Many of these effects appear to occur even in mild-to-marginal iron-deficient states in animal studies. However, as discussed above, there is still little information available on whether mild or moderate iron deficiency influences immune effects in humans (Weinberg, 1992).

2.8.1.2 Mechanisms for the importance of iron to the immune system

The molecular and cellular mechanisms responsible for immune changes during iron deficiency are complex and remain unclear. This is because iron is important in several crucial, metabolic pathways in immune cells. Knowledge concerning the roles of iron and iron-binding proteins in lymphocyte physiology and pathology has developed rapidly over the last few years. The genes for the major iron-binding proteins have been cloned and sequenced and are now being studied regarding transcriptional and posttranscriptional regulatory mechanisms in T cells, B cells, macrophages, and NK cells (Uchida, 1994).

2.9Zinc (Zn)

Zinc is a dietary trace mineral that, in addition to its many essential functions in growth and development, is essential for the function of cells of the immune system. Zn is required for the activity of more than 100 enzymes associated with carbohydrate and energy metabolism, protein degradation and synthesis, nucleic acid synthesis, heme biosynthesis and CO_2 transport (Kumari and Chandra, 2003). Zn deficiency impedes host-defense systems, leading to increased susceptibility to a variety of pathogens and a deficiency of Zn is known to occur in many diseased states that involve the immune system (Fraker, 2000), these include alcoholism, renal disease, burns, and gastrointestinal tract disorders as well as HIV and diarrhea (Prasad *et al.*, 1998). The genetic Zn malabsorption syndrome,

acrodermatitisenteropathica, is associated with frequent severe infections with fungi, viruses, and bacteria with concomitant thymic atrophy, energy reduced proliferative response, decreased T-helper cells, and deficient thymic-hormone activity (Prasad *et al.*,2000).

2.9.1 Clinical and Experimental Evidence for the Essentiality of Zinc

A number of experimental trials have examined the ability of Zn to improve immune function during various diseases. For example, Zn supplementation resulted in a reduced duration and severity of cold symptoms (Prasad *et al*., 2000). However, other studies have shown that Zn compounds appear to have limited effectiveness for common cold treatment and a meta-analysis of eight published, randomized clinical trials showed that the evidence for effectiveness of Zn salts lozenges in reducing the duration of common colds is still lacking (Jackson *et al.,* 2005). In patients with sickle-cell disease, Zn supplementation increased IL-2 production, decreased incidence of bacteriologically positive infections, decreased the number of hospitalizations, and decreased the number of vasoocclusive pain crises (Prasad *et al.,* 1998). In young children, Zn supplementation reduced diarrhea duration, pneumonia, growth-stunting, acute lower respiratory infections and morbidity, (Sazawal*et al.,* 1998). Children receiving Zn supplementation had a significantly higher proportion of CD4+CD3+ cells (CD3, CD4, and CD4/CD8 ratio) in peripheral blood and improved T-cell-mediated immunity (CMI) (Sazawal*et al.*, 1997).

Animal studies have confirmed that Zn deficiency is associated with a significant reduction in T-helper cell function (Jackson *et al.,* 2005), impaired DTH responses, compromised B-cell development, low IgG production, decreased NK lytic activity and increased mortality to various infectious organisms (Frake*et al*., 1982). Maternal Zn deprivation results in

offspring with reduced thymus and spleen size, splenocyte numbers, mitogen responses and antibody production. However, the poor Ab-mediated response capacity and defective DTH could be restored by Zn supplementation (Fraker*et al*., 1984). It is interesting that the effects of Zn deficiency may be immune cell-type specific, because one study suggests that myeloid cell numbers and function are not compromised by such a deficiency (Wirth *et al*., 1984).

2.9.2Proposed mechanisms for the immune essentiality of Zinc

The proposed mechanisms by which Zn influences immune functions include generation of oxygen radicals, lymphocyte maturation, cytokine production, and the regulation of apoptosis and gene expression (Fraker *etal*., 2000).

During Zn deficiency, the presence of higher proportions of granulocytes (as much as 50%) and monocytes (almost twofold) suggest that the myelopoietic environment of the marrow is more protected from or even up-regulated during Zn deficiency (Frakerand Tellford,2007). Although the numbers may not be compromised, the function of these cells may be. Zn-dependent enzymes or reactions are involved in the generation of oxygen radicals and suboptimal levels of Zn have been demonstrated to lower the killing ability of internalized parasites by macrophages (Wirth *et al*., 1989).

Furthermore, the capacity of macrophages to engulf and kill parasites can be restored after treatment with Zn. Whether impaired killing ability of macrophages is a result of decreased production of H_2O_2 or of another Zn-related function or process remains to be established (Wirth*etal.,* 1989)

The decreased cell-mediated immune functions and the increased frequency of infection in Zn-deficient subjects may be linked to the effects of Zn on cytokine production (decreased IL-2 production), a decrease in CD4+/CD8+ cell ratio, and a decrease in the production of antigen mature CD4+CD45R0+ cells, suggesting an effect on T-helper cell maturation . Zn influences the activity of multiple enzymes at the basic level of replication and transcription (Prasad *et al*., 2000) For example, Zn is needed for the activity of thymidine kinase during the S-phase of cell growth and for the activation of the Zn finger protein NF-κB that is involved in IL-2 and IL-2R expression. In Zn-deficient cells, the activation, translocation, and binding of NF-κB to DNA are inhibited NK activity and cytotoxic T-cell precursors (CD8+CD73+) are decreased with Zn restriction which may be linked to decreased IL-2 production. Zn deficiency is also associated with an increase in plasma corticosterone, which can contribute to T-cell immunosuppression (DePasquole and Fraker., 1979).

Some of the changes in T-cell maturation and function observed during Zn deficiency are likely related to decreases in Zn-dependent thymulin activity. Zn deficiency in experimental animals results in atrophy of thymic and lymphoid tissue (Prasad *et al*., 1988) with losses of precursor T and B cells in the bone marrow. This is demonstrated by the dose-related decline in the number of pre-B cells (B220+), immature B cells (B220+IgM+IgD−) and mature B cells (IgM+IgD+) (King *et al*., 1995).

In vitro, low concentrations of Zn have been shown to induce apoptosis in mouse CD4+CD8+ thymocytes, whereas high zinc concentrations have been shown to block apoptosis In vitro, high concentrations of Zn blocked apoptosis by preventing activation of the endonuclease, which is involved in DNA fragmentation and inhibited steroid binding (possibly by binding to the vicinal cysteines in the receptor-ligand-binding site) to the

glucocorticoid receptor during glucocorticoid-induced apoptotic death (Fraker and Telford., 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Biological Microscope (BOECO Germany, MB-300),CareSart^{TM} Malaria HRP2 (Pf), 3.0% Giemsa stain. Auto-Hematology Analyzer (Mindray, BC-3600).Semi-automated Chemistry-Analyzer (SURECHEM-Version, 2012). ELIZA kits (CRP, SAA, Ferritin, Transferrin), Micropipettes, Multi-channel pipette and disposable tips. Micro plate reader (GF- M 3000), B. Bran Scientific & Instrument Company England.

3.1.1Reagents

All Reagents used (Water- soluble pyridylazo dye, Color 3, 5-DiBr- PAESA, Ferro zine, M-30R, M-30D) were purchased from reputable Companies, these includes: Randox Laboratories limited, UK BT29 4QY and Shenzhen Mindray Biochemical/ Electronic CO. LTD, Nanchang- Shenzhen 518057 P. R. China.

3.1.2Study Area

The study was conducted in three (3) Primary Health Care centers (PHCs), within Samaru town in SabonGari Local Government Area, Kaduna State. The centres are: University Health Services (Sick Bay), Ahmadu Bello University, Samaru- Zaria,Primary Health Care Centre (Central) Samaru- Zaria and Primary Health Care Centre (Hayin Dogo) Samaru-Zaria.

The research was conducted within the period of four months (March, 2015 -June, 2015).

3.1.3Sample Size

The survey design was based on a simple random sampling; the sample size required was calculated according to the following formula (Naing*et al*., 2006):

$$n = \frac{t^2 \times p \times q}{c^2}$$

Where:

n= required sample size

t= confidence level at 95% (standard value =1.96)

p= estimated prevalence of maternal mortality rate due to malaria.

q= Complementary probability (1- p) = 0.89

c= margin of error at 5% (standard value= 0.05)

Therefore,

$$n = \frac{(1.96)^2 \times 0.11 \times 0.89}{(0.05)^2}$$

$$n = \frac{3.842 \times 0.11 \times 0.89}{0.0025}$$

$$n = \frac{0.3761}{0.0025}$$

n= 150.44

3.1.4Study Population

The study population comprised of 75 pregnant women with *P. falciparum*and four hundred and seventy two (472) pregnant women without malaria; matched for age and stage of

pregnancy, chosen from the population of consented pregnant women attending ante-natal clinics in the three (3) Primary Health Centers.

3.1.4.1Inclusion Criteria

- Pregnant women with malaria parasitemia (Subject)
- Pregnant women without malaria parasitemia (Control)
- Malaria infection due to *P. falciparum* only

3.1.4.2 Exclusion Criteria

- Pregnant women with Hepatitis B or C positive
- Pregnant women with abnormal Liver function
- Malaria parasitemia due to other species or mixed infections

3.1.5Ethical Approval

Ethical approval was obtained from the Ethics and Research Committee of University Health Services, AhmaduBello University, Zaria (Appendix VI) and also from Health DepartmentSabonGari Local Government Area Kaduna State (Appendix VII), while consent were sought from the subjects through the administration of consent form (Appendix VIII).

3.2 Methods

3.2.1Data Collection

Data were collected from various Health Centers with the help of pretested semi structured questionnaire to document their socio-demographic characteristics (Appendix I). Adopted from Demographic & Health Survey (DHS) model questionnaires- phase 6 (2008- 2013, *French & English*).

3.2.2 Anthropometric Measurements

Maternal mid-upper arm circumference (MUAC) is a potential indicator of maternal nutritional status. It could be used in association with other anthropometric measurements. Weight was taken using a weighing scale and height measured with the aid of a stadiometer (WHO, 1995).

3.2.3Laboratory Analysis

3.2.3.1Sample Collection

Exactly 5ml of blood samples was collected intravenously by venipuncture from the ante-cubital vein of the forearm after thorough sterilization of the site with 70 % alcohol and swab, using sterile needle and syringe. Two (2ml) of the blood was delivered into clean EDTA bottle (Cheesbrough, 1998).

3.2.3.2 Haematological Parameters

Mindray (BC-3600),Full automated haematology analyzer was used for hematological assessment ofHb- estimation, White blood cells and Platelets counts.

3.2.3.3 Assessment of Plasmodium falciparum Infection

Microscopic method was used to screen subjects' blood sample for malaria parasite. Thin blood films of each sample was prepared and stained in 3% Giemsa solution, as described by Davey (1986). The stained slides were then examined using x100 objectives in oil immersion.

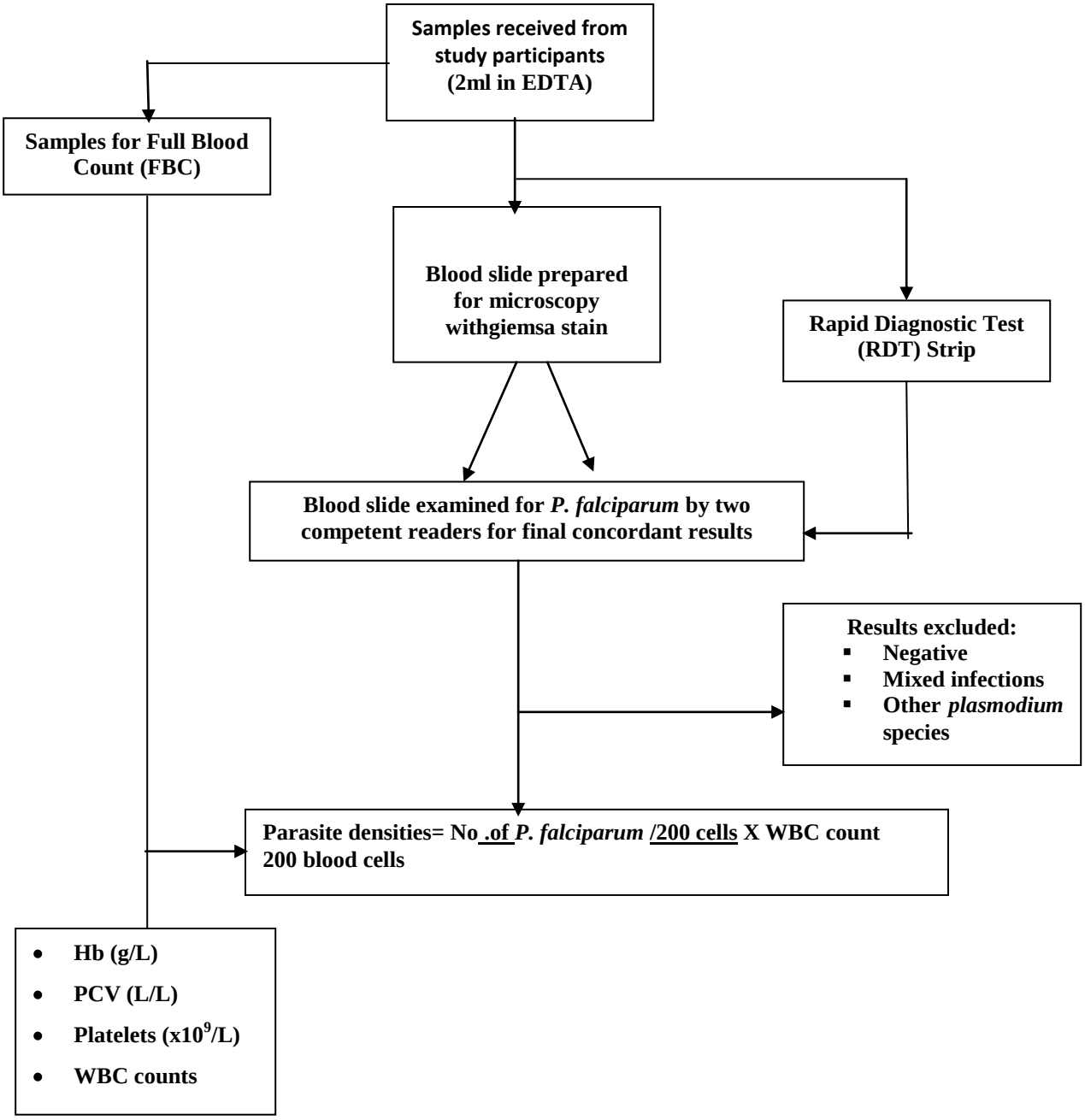


Figure 3.1:Experimental Design for *P. falciparum* screening parasite density and some Hematological parameters

3.2.4Biochemical procedure

Semi-auto Analyzer (SURECHEM, 2012) was used to determine the serum concentrations of Zinc, Copper, Albumin and Iron spectrophotometrically.

3.2.4.1Determination of Zinc using Water- soluble pyridylazo dye(1984)

Principle: Zinc forms with 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)-phenol a red chelate complex. The increase of absorbance can be measured and is proportional to the concentration of total zinc in the sample.

Procedure:

Three sets of clean dry test tubes were arranged accordingly in a test tube rack. These includes tubes for test samples, Blank and Standard.Into each of the tubes, 1000µl of working reagent was dispensed including blank and standard tubes.Fifty microliter (50µl) of each sample was pipetted and dispensed into corresponding labelled tube, while blank tube contained working reagent only.Each content was mixed and incubated at 37^{o}C for 5min.Absorbance of each sample, blank and standard were taken at 560nm wavelength.

Calculation:

$A_{(S)} = A_{(Sample)} - A_{(RBL)}$

$A_{(STD)} = A_{(Standard)} - A_{(RBL)}$

$Conc_{(STD)}$

$$Conc_{(Sample)} = \frac{A_{(Sample)} - A_{(RBL)}}{A_{(Standard)} - A_{(RBL)}} x Conc_{(STD)}$$

Conversion Factor: ug/dL x 0.153 = umol/L

Reference Values

Adults:46 – 150µg/dl (7 – 23µmol/L)

*3.2.4.2 Determination of Copper using Color 3, 5-DiBr- PAESA(*Abe and Noma, 1989*)*

Principle:Colorimetric test without deproteinization of the sample end point increase. At pH 4.70 in a buffered media, copper is released from ceruloplasmine complex and forms with the specific complexant 3-5 DiBr-PAESA a stable coloured complex. The colour intensity is proportional to the amount of copper present in the sample.

Procedure:

Three sets of clean dry test tubes were arranged accordingly in a test tube rack. These includes tubes for test samples, Blank and Standard.Into each of the tubes, 1000µl of working reagent was dispensed including blank and standard tubes.Fifty microliter (50µl) of each sample was pipetted and dispensed into corresponding labelled tube, while blank tube contained 50µl of distilled water. Each content was mixed and incubated at 37^{o}C for 5min.Absorbance of each sample, blank and standard were taken at 582nm wavelength.

Calculation

$\frac{(A_2\text{-}A_1)\ \text{sample}}{(A_2\text{-}A_1)\ \text{standard}} \times 100\ (\text{standard concentration}) = \mu g/dl\ \text{copper in the sample}$

Conversion Factor: ug/dL x 0.1573 = umol/L

Reference Values

Male:	80-155µg/dl (12.9µmol/L – 24.4µmol/L)
Female:	70-140µg/dl (11.0µmol/L- 22.0µmol/L)

3.2.4.3Determination of Albumin usingBromo Cresol Green

Principle: Albumin in the presence of bromocresol green at slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue. The intensity of the colour formed is proportional to the albumin concentration in the sample(Silverman*et al.,* 1986).

Procedure:

Three sets of clean dry test tubes were arranged accordingly in a test tube rack. These includes tubes for test samples, Blank and Standard.Into each of the tubes, 1000µl of working reagent was dispensed including blank and standard tubes.Five microliter (5µl) of each sample was pipetted and dispensed into corresponding labelled tube, while blank tube contained working reagent only.Each content was mixed and incubated at room temperature for 5min.Absorbance of each sample, blank and standard was taken at 630nm wavelength.

Calculations

$$\text{Albumin (g/dL)} = \frac{\text{(A) Sample}}{\text{(A)Standard}} \times 5 \text{ (calibrator concentration)}$$

Conversion Factor g/dL x 144.9 = umol/L

Reference Value:30– 52 g/l

3.2.4.4 Determination of Iron usingFerro zine (Iron –FerroZineMethod, 1980).

Principle: The iron is dissociated from transferrin-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent from by means of ascorbic acid. Ferrous ions give with Ferro Zine a coloured complex:

$$\text{Transferrin } (Fe^{3+})_2 + e^- \xrightarrow{\text{Ascorbic acid}} 2Fe^{2+} + \text{Transferrin}$$

$$Fe^{2+} \xrightarrow{\text{Ferro Zine}} \text{Colored complex}$$

The intensity of the colour formed is proportional to the iron concentration in the sample.

Procedure:

Three sets of clean dry test tubes were arranged accordingly in a test tube rack. These includes tubes for test samples, Blank and Standard. Into each of the tubes, 1000µl of working reagent was dispensed including blank and standard tubes. Two hundred microliter (200µl) of each sample was pipetted and dispensed into corresponding labelled tube, while blank tube contained 200µl of distilled water. Each content was mixed and incubated at 37^{o}C for 5min. Absorbance of each sample, blank and standard was taken at 562nm wavelength.

Calculations:

$$\text{Iron (µg/dL)} = \frac{\text{(A) Sample - (A) Sample Blank}}{\text{(A) Standard}} \times 5 \text{ (calibrator concentration)}$$

Conversion Factor ug/dL x 0.179 = umol/L

Reference Values:

Male: 65-175µg/dl (11.6-31.3µmol/L)

Female: 50-170µg/dl (9.0-30.4µmol/L)

3.2.5Quantitative Determinations of C-reactive proteins (CRP), Serum amyloid A (SAA), Ferritin and Transferrin using Enzyme–immunosorbent assay (ELISA) Test kit.

3.2.5.1 C - reactive protein(CRP) Concentration using 'Perfemed' ELISA Test Kit(Catalog No. 10603)

Procedure

Patient and control serum were diluted 100 fold prior to use anddesired number of coated wells were secured in the holder.Undiluted CRP standards, diluted specimens, and diluted controls were dispensed (50µL) each into appropriate wells.Fifty microliter (50µL) of sample diluent was dispensed into each well, and thoroughly mixed for 30 seconds and was incubated at room temperature (18-22^{o}C) for 30 minutes. The mixture was removed by flicking the plate contents into a waste container. Microtitre wells were rinsed and flicked 5 times with washing buffer (x1). Wells were strike sharply onto absorbent paper to remove all residual water droplets. Then,CRP enzyme conjugate reagent was dispensed (100µL) into each well,thoroughly mixed for 30 seconds and incubated at room temperature (18-22^{o}C) for 30 minutes. After which incubation mixture was removed by flicking plate contents into a waste container and microtitre wells rinsed and flicked 5 times with washing buffer (x1). The wells were strike sharply onto absorbent paper to remove all residual water droplets and TMB reagent was dispensed (100µL) to each welland gently mixed for 5 seconds. The micro plate was then incubated at room temperature for 30 minutes andreaction was stopped by adding 100µL of stop solution to each well. Microtitre well reader was used within 15 minutes to read Absorbance at 450nm.

Calculations

Using the mean absorbance value for each sample, corresponding concentrations of CRP (ng/mL) were determined from the standard curve.

Reference Values: Adult Serum: 68 - 8200ng/mL.

3.2.5.2 Ferritin concentration using Ferritin Test System Product Code. 2825-300 (Monobind Inc. Lake Forest, CA 92630, USA)

Principle: The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzymes and immobilized), with different and distinct epitome recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a micro plate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti- ferritin antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the micro wells resulting in immobilization of the complex. After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a micro plate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure

Micro plate's wells for each serum references, control and patient specimen were formatted and assayed in duplicate by pipetting 25µL of the appropriate serum reference, control or specimen into the assigned well.Ferritin biotin reagent was added to each well (100µL) and micro plate Swirled gently for 30 seconds, then incubated for 30 minutes at room temperature.The contents of the micro plate was discarded by aspiration and the plate was blotted with absorbent paper.Manual plate washer was used to wash the micro plates following the manufacturer's instruction for proper usage. Ferritin enzyme conjugate (100µL) was added to each well and was incubated for 30 minutes at room temperature.The contents of the micro plate was discarded by decantation and 300µL of wash buffer was used as above (4). The washing was repeated (tap and blot) three times. Working substrate solution (100µL) was added to all wells and incubated at room temperature for fifteen (15) minutes followed by addition of 50µL of stop solution to each well with gentle mixing for 20 seconds.Microtitre well reader was used within 15 minutes to Read absorbance at 450nm.

Calculation

Using the mean absorbance value for each sample, corresponding concentrations of Ferritin (ng/mL) were determined from the standard curve.

Reference Values:

Males = 16-220ng/ml

Females = 10-124ng/ml.

ng/ml x 2.247 = pmol/L

3.2.5.3 Human Transferrin (TRF) concentration using'Perfemed' ELISA Test Kit Catalog No. 10717.

Principle: The kit assay human TRF level in the sample, use purified human TRF antibody to coat microtitre plate wells, make solid-phase antibody, then added was TRF to the wells, combined TRF which with enzymes labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, added was substrate, which becomes blue color at HRP enzyme-catalyzed, reaction was terminated by the addition of sulphuric acid solution and the color change was measured sprectrophotometrically at a wavelength of 450nm. The concentration of TRF in the samples was then determined by comparing the absorbance of the samples to the standard curve.

Procedure

Serial dilution of standard on the coated microtitre plate was prepared in order to obtain a standard curve (density: 4.8ng/ml, 3.2ng/ml, 1.6ng/ml, 0.8ng/ml, and 0.4ng/ml).Sample diluted to 40µL was added to the sample well followed by 10µL of serum sample. The content was gently mixed and incubated for 30 minutes at 37^{o}C.Incubation mixture was removed by aspirating contents of the plate into a sink using a squirt bottle and each well was completely filled with wash solution, then aspirated into a sink. This procedure was repeated four more times to give a total of five washes. After final wash, the plate was inverted, and later blotted using absorbent paper until no moisture appears and Fifty microliter (50µL) enzyme conjugate reagent was added to each well, except blank well with content was gently mixed and incubated for 30 minutes at 37^{0}C. Incubation mixture was removed by aspirating contents of the plate into a sink using a squirt bottle with each well completely filled with wash solution, then aspirated into a sink. This procedure was repeated four more times to give a total of five washes. After final wash, the plate was

inverted, and later blotted using absorbent paper until no moisture appears.Fifty (50µL) substrate A and substrate B were added to each well, then covered and incubated for 15 minutes at 37^{o}C. After which 50µL stop solution was added to each well and gently mixed.Microtitre well reader was used within 15 minutes to read absorbance at 450nm.

Calculations

Using the mean absorbance value for each sample, corresponding concentrations of TRF (ng/mL) were determined from the standard curve.

Reference Values:

0.1ng/ml – 5ng/ml

3.2.5.4Human Serum AmyloidA (SAA) concentration using'Perfemed' ELISA Test Kit (Catalog No. 10735)

Principle: The kit assay human SAA level in the sample, use purified human SAA antibody to coat microtitre plate wells, make solid-phase antibody, then added was SAA to wells, combined SAA with enzymes labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, add substrate which becomes blue color at HRP enzyme-catalyzed, reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm. The concentration of SAA in the samples was then determined by comparing the absorbance of the samples to the standard curve.

Test Procedure:

Patient serum and control serum were diluted 100 fold prior to use.Desired number of coated wells were secured the in the holder.Undiluted SAA standards, diluted specimens,

and diluted controls were dispensed (50µL) each into appropriate wells.Fifty (50µL) of sample diluent was dispensed into each well, and thoroughly mixed for 30 seconds. Mixture was incubated at room temperature (18-22^{o}C) for 30 minutes.The incubation mixture was removed by flicking the plate contents into a waste container. Microtitre wells were rinsed and flicked 5 times with washing buffer (x1).Wells were strike sharply onto absorbent paper to remove all residua water droplets.SAA enzyme conjugate reagent was dispensed (100µL) into each well.Thoroughly mixed for 30 seconds. Then incubated at room temperature (18-22^{o}C) for 30 minutes.The incubation mixture was removed by flicking plate contents into a waste container. Microtitre wells were rinsed and flicked 5 times with washing buffer (1x).The wells were strike sharply onto absorbent paper to remove all residual water droplets.The micro plate was incubated at room temperature for 30 minutes.Reaction was stopped by adding 100µL of Stop Solution to each well.Microtitre well reader was used within 15 minutes to Read absorbance at 450nm.

Calculation

Using the mean absorbance value for each sample, corresponding concentrations of SAA (ng/mL) were determined from the standard curve.

Reference Values*:*

30µg/L – 800µg/L

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *P. falciparum* Infection in Pregnant Women by HealthCare Facility

Out of the 547 pregnant women examined for malaria parasite within the period of four months (March, 2015-June, 2015), those found positive were 75 (13.7%) while 472 (86.3%) were negative for malaria infection. *Plasmodium falciparum* was the only malaria parasite considered in the blood sample for this study. Malaria prevalence rate according to Healthcare centers are shown in Table 4.1: The Hayindogo PHC has the highest (17.9%), compared to the lowest in University Health Center (10.6%).

4.2 Distribution of Pregnant Women Infected with *P. falciparum*Based on Socio-Demographic Characteristics.

Malaria parasites were more prevalent among subjects between the groups of 15-19 years (17.5%) and 20-24 years (15.6%) with the mean parasite densities of 5925 and 5966 parasite/µl of blood respectively; as compared to the older pregnant mothers of ages 30-34 years (11.8%) and 35-39 years (10.9%) with mean parasite densities of 2478 and 1459 parasite/µl of blood respectively (Table 4.2).There was significant difference among the age groups.

Full time housewife (354) was the highest occupation of pregnant women in the study and there was no difference ($p > 0.05$) in terms of infection rate as well as the malaria parasite density between the occupational groups (Table 4.2).

Majority of pregnant women had secondary education as their minimum qualifications (182), with lower infection (13.7%) and mean parasite density of 2933/µl of blood, compared to No-Formal education with higher infection rate (17.4%) and mean parasite

density of 4419/µl blood (Table 4.2).There was significant difference between the categories of educational status.

Most of pregnant women live off-campus (438) with higher infection rate of 14.6% and mean parasite density of 4739/µl blood. The least were those living On-campus with 10.0% and mean parasite density of 4120/µl blood (Table 4.2). The difference was not significant.

4.2.1 Use of Insecticides Treated Nets (ITNs) by Pregnant Women

Majority of the pregnant women under study 226 (41.6%) did not use ITNs at all during pregnancy; while those with ITNs but not in use always 146 (26.7%) were the least (Figure 4.1).

4.2.2 Use of IPTp by the Pregnant Women and frequency of malaria infection

Majority of pregnant women 376 (68.7%) complied with the policy of Intermittent Preventive Treatment of malaria in pregnancy (IPTp) by taking complete dose of Sulfadiazine Pyrimethamine (SP) drug in the second trimester of pregnancy (Figure 4.2). Most of the pregnant women 284 (52.0%) used to have malaria attack when not pregnant (Figure 4.2).

Table 4.1: Prevalence of Malaria Parasite (*Plasmodium falciparum*) Among Pregnant Women by Health Care Facility

Health Care Facility	Number Examined	Number Infected (%)	Number Not-Infected (%)
University Health Centre	235	25 (10.6)	210 (89.4)
Primary Health Center Samaru	172	25 (14.5)	147 (85.5)
Primary Health Center H/Dogo	140	25 (17.9)	115 (82.1)
Total	**547**	**75 (13.7)**	**472 (86.3)**

Table 4.2: Distribution of *P. falciparum* Infected Pregnant Women Based on Socio Demographic Characteristics

Demographic. Characteristic.	Number Examine	UHC	HCS	HCHD	% Infected	Mean MPD ±SD
Age (Yrs.)						
15-19	80(14)	17(2)	40(7)	23(5)	17.50	5925± 106
20-24	179(28)	81(9)	53(8)	45(11)	15.60	5966±136
25-29	113(13)	60(4)	23(3)	30(6)	11.50	3635±126
30-34	102(12)	38(3)	37(5)	27(4)	11.80	2478±72
35-39	55(6)	28(3)	15(2)	12(1)	10..9	1459±26
≥ 40	18(2)	11(1)	4(0)	3(1)	11.10	3931±70
Total	**547(75)**	**235(25)**	**172(25)**	**140(25)**	**13.70**	**-**
Occupation						
Civil servant	47(7)	29(4)	7(1)	11(2)	14.90	4552±97
Full H/Wives	354(50)	173(18)	87(13)	94(19)	14.10	4366±103
Petty Trading	124(18)	30(3)	66(11)	28(4)	14.50	4811±146
Full time Business	22(0)	3(0)	12(0)	7(0)	0	0.0
Total	**547(75)**	**235(25)**	**172(25)**	**140(25)**	**13.70**	**-**
Education						
N/Formal Education	69(12)	10(3)	21(5)	38(4)	17.40	4419±108
1^o education	165(23)	46(3)	67(9)	52(11)	13.90	4942±115
2^o education	182(25)	87(11)	50(5)	45(9)	13.70	2933±94
3^o education	131(15)	92(8)	34(6)	5(1)	11.50	2586±131
Total	**547(75)**	**235(25)**	**172(25)**	**140(25)**	**13.70**	**-**
Place of residence						
On-campus	109(11)	79(7)	24(3)	6(1)	10.10	4120±113
Off- campus	438(64)	156(18)	148(22)	134(24)	14.60	4739±92
Total	**547(75)**	**235(25)**	**172(25)**	**140(25)**	**13.70**	**-**

Values in brackets are the number infected, MPD = Malaria Parasite Density. SD = Standard Deviation, %= percent,UHC = University Health Centre, HCS = Health Center Samaru, HCHD= Health Center H/Dogo

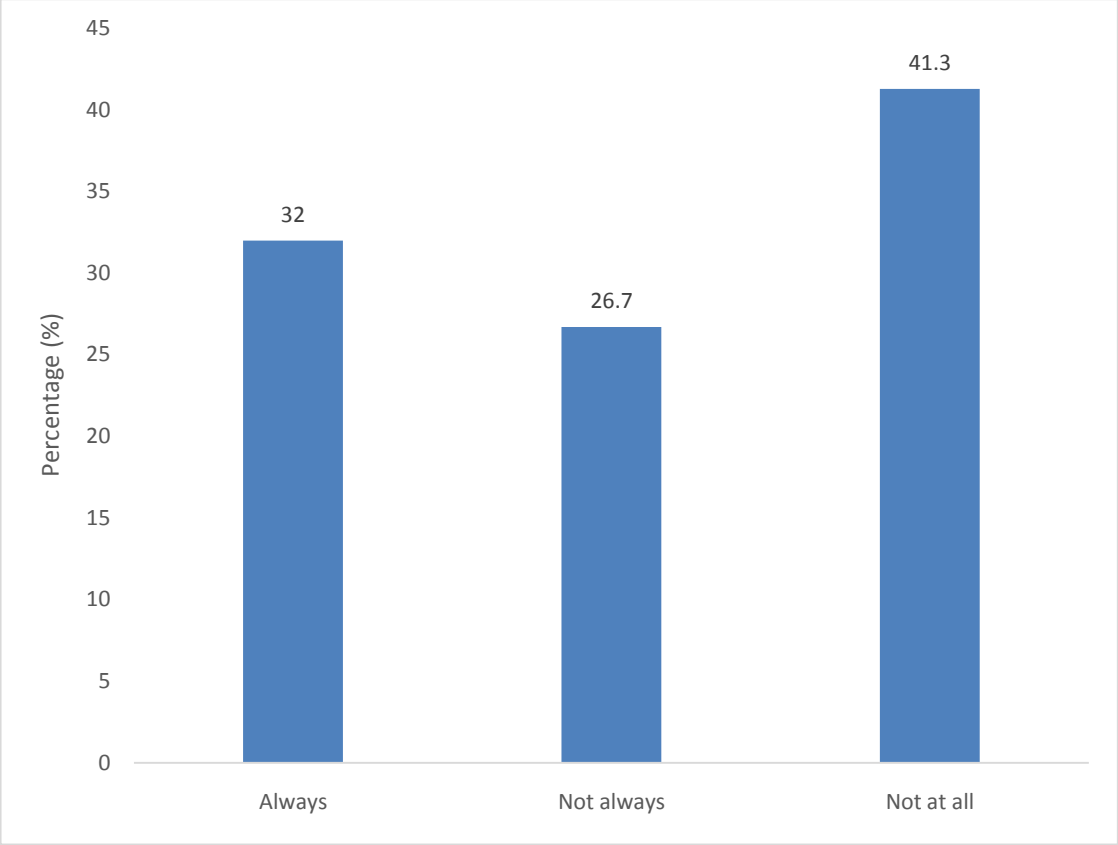


Figure 4.1: Use of Insecticides Treated Nets (ITNs) byPregnant Women attending Health Care Facilities in Samaru

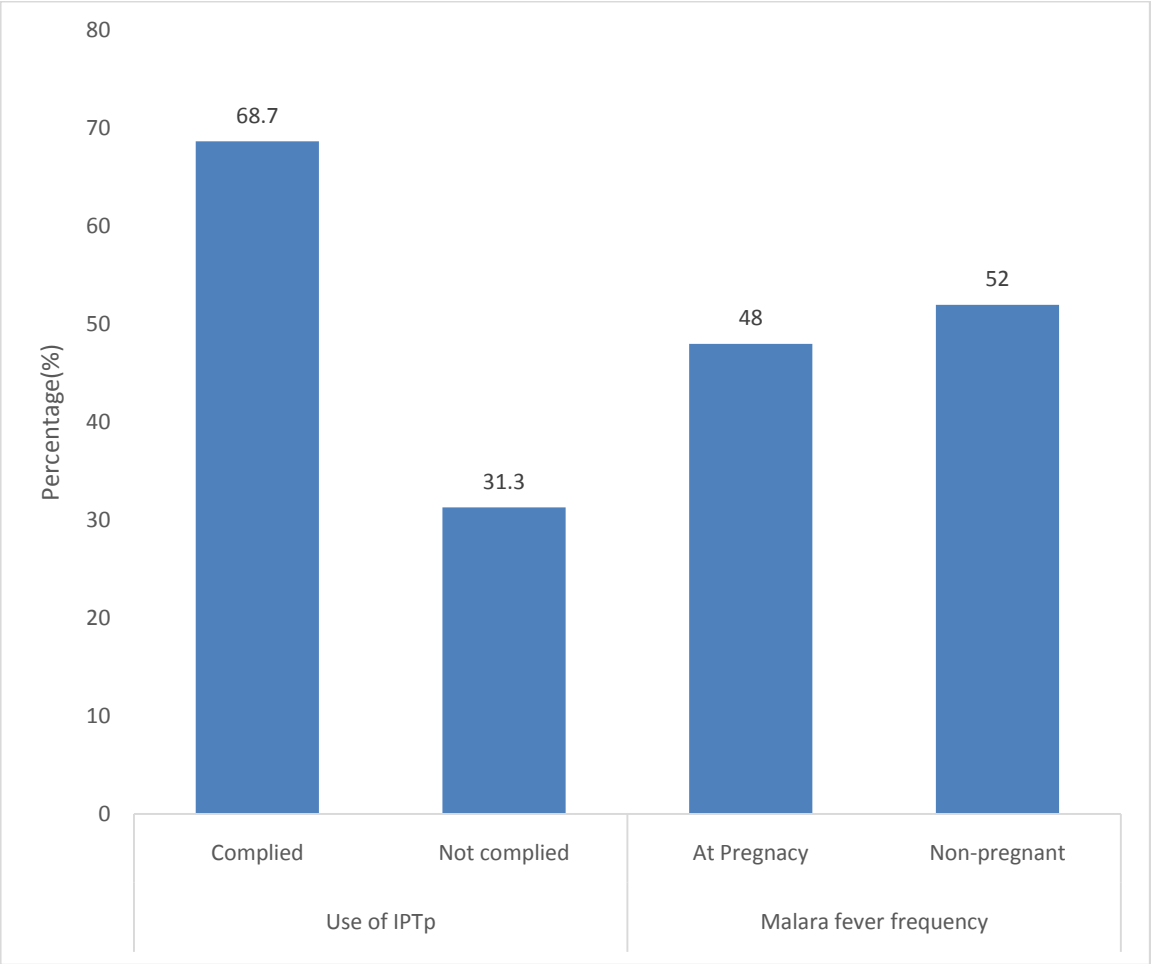


Figure 4.2: The use of IPTp by the Pregnant Women and frequency of malaria infection

4.3.Relationship between malaria parasite density, parity and gestation period

Highest prevalence of 19.9% with mean parasite density of 7644/µl blood was observed among women in the first trimester of pregnancy, followed by 14.5% with mean parasite density of 4671/µl blood in the second trimester, least was observed in the third trimester 8.7% with mean parasite density of 1630/µl blood (Table 4.3).

Pregnant women of primigravid with 19.0% infection rate and mean parasite density burden of 6760/µl blood was highest while multigravida infection rate was 7.7% and malaria parasite density of 3442/µl as the least (Table 4.3).

4.3.1Birth Spacing between last and current pregnancy among Pregnant Women

Majority of the respondents 233 (42.7%) practiced child-spacing of at least 3 years duration before the next conception, while the least among them 51 (9.3%) practiced one year duration (Figure 4.3).

Table 4.3: Relationship between malaria parasite density, parity and gestation period

Variable	**Number Examined**	**No. Infected**	**% Infected**	**Mean MPD±SD**
Gestation Period				
1st Trimester	146	29	19.90	7644±143
2nd Trimester	193	28	14.50	4671±122
3rd Trimester	208	18	8.70	1630±49
Total	**547**	**75**	**13.70**	**-**
PARITY				
Primigravid	237	45	19.00	6760±129
Second gravidae	141	17	12.10	4036±101
Multi gravidae	169	13	7.70	3442±89
Total	**547**	**75**	**13.70**	**-**

MPD = Malaria Parasite Density. SD = Standard Deviation,

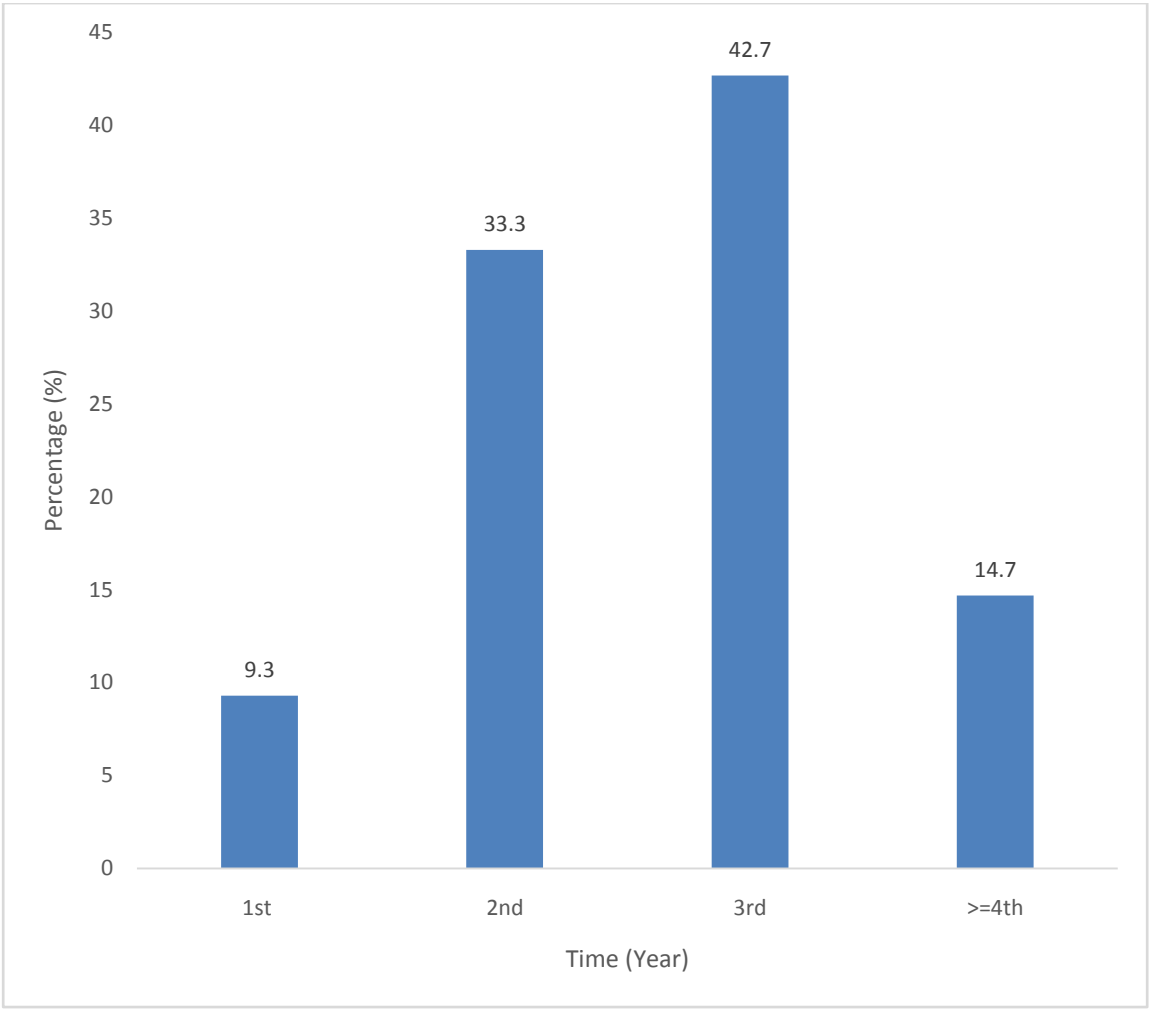


Figure 4.3: Birth Spacing between last and current pregnancyamongPregnant Women

4.4 Indices of Iron Status among Pregnant Women with *Plasmodium falciparum* Infection

Ferritin and transferrin concentrations weresignificantlyhigher ($P<0.05$) among pregnant women with malaria and Iron deficiency anemia as compared to pregnant women without malaria and those with malaria without Iron deficiency (Table 4.4).

4.5 Nutritional Status of Pregnant Women with and Without *Plasmodium falciparum* Infection

There was no statistical difference in MUAC measurements between the control group and those infected with malaria (*P. falciparum*). Platelets counts, Zn and Cu concentrations did not show any significant difference but the value of Zn/Cu ratio in pregnant women with malaria was lower and significant ($P<0.05$) than in pregnant women without malaria (control) (Table 4.5)

Table 4.4: Relative Values of Indicators of Iron Status among Pregnant Women attending Health Care Facilities in Samaru, Zaria.

Indicators of iron status	**Pregnant Women without Malaria (control) (n = 75)**	**Malaria without Iron Deficiency Anemia (n = 50)**	**Malaria with Iron Deficiency Anemia (n = 25)**
Fe (µmol/L)	19.0 ± 7.0^{a}	16.9 ± 6.3^{a}	7.6 ± 1.0^{b}
Hb (g/L)	111.0 ± 19.0^{a}	121 ± 10.1^{a}	92 ± 10.4^{b}
Ferritin (pmol/L)	140.0 ± 82.7^{a}	151.9 ± 72.8^{b}	385.8 ± 56.6^{c}
Transferrin (g/L)	3.80 ± 0.52^{a}	3.30 ± 0.39^{a}	7.7 ± 0.26^{b}

Values with different superscript(a,b,c) across the row are significantly ($P<0.05$) different.

Mean ± SD values**.**

Table 4.5: Relative Nutritional Status of Pregnant Women With and Without *Plasmodium falciparum* Infection.

Parameters	**Pregnant women without malaria (control) (n=75)**	**Pregnant women with malaria (n=75)**
MUAC (mm)	261 ± 20[a]	266 ±21[a]
Platelets ($x10^9$/L)	210.0 ± 76.4[a]	208.0 ±65.3[a]
Zn (µmol/L)	13.1 ± 3.0[a]	12.1 ± 4.0[a]
Fe (µmol/L)	19.0 ± 7.0[a]	14.0 ± 6.9[b]
Cu (µmol/L)	17.2 ± 4.8[a]	18.8 ± 5.2[a]
Zn/Cu	0.76[a]	0.64[b]

Values with different superscript (a,b,c) across the row are significantly ($P<0.05$) different.

Mean ± SD values.

4.6.Serum C-reactive protein and Malaria parasite density of *P. falciparum* Infected Pregnant Women attending PHCs in Samaru Zaria

The strength of relationship between C - reactive protein (CRP) concentration and malaria parasite*(P. falciparum)* density was linear, very strong (R^2=0.7) and highly significant (P<0.01); despite the presences of some rogue points (Figure 4.4).

4.6.1Effect of Malaria Parasite Density (MPD) on Serum Amyloid A of *P. falciparum* Infected Pregnant Women attending PHCs in Samaru Zaria.

The strength of relationship between Serum Amyloid A (SAA) and malaria parasite density (MPD) was linear, strong (R^2=0.3) and highly significant (P<0.01); despite the presences of some rogue points (Figure 4.5).

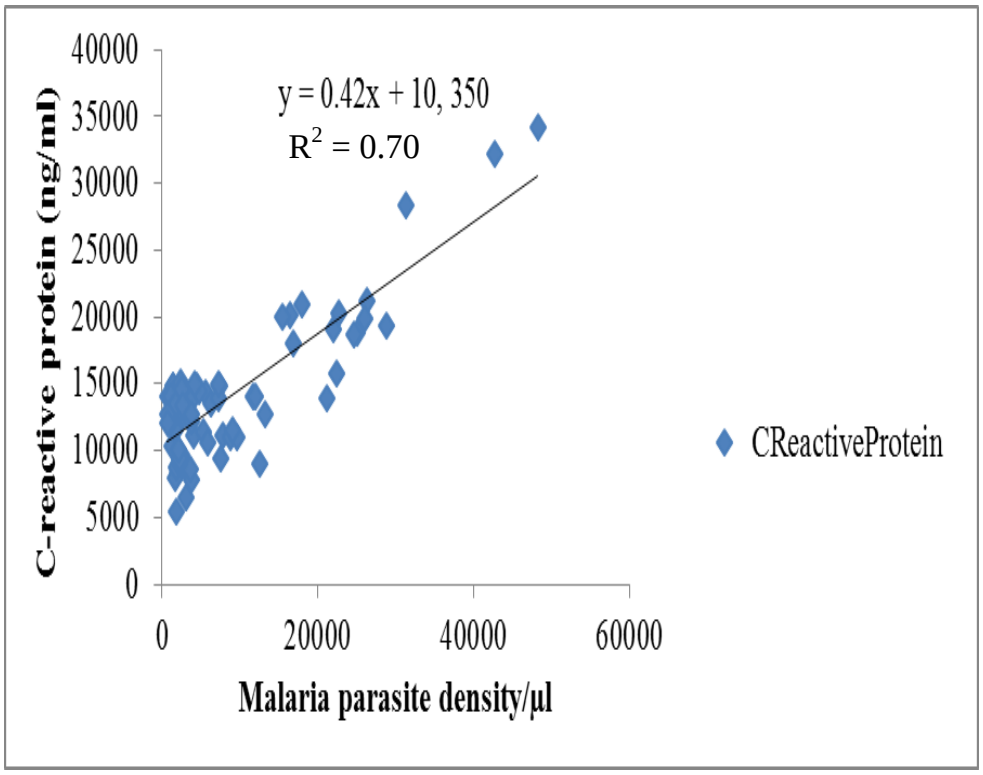


Figure 4.4: Scatter plot showing the relationship between C-reactive protein Concentration and Malaria parasite (*P. falciparum*) density in Infected Pregnant Women attending Three PHCs in Samaru, Zaria

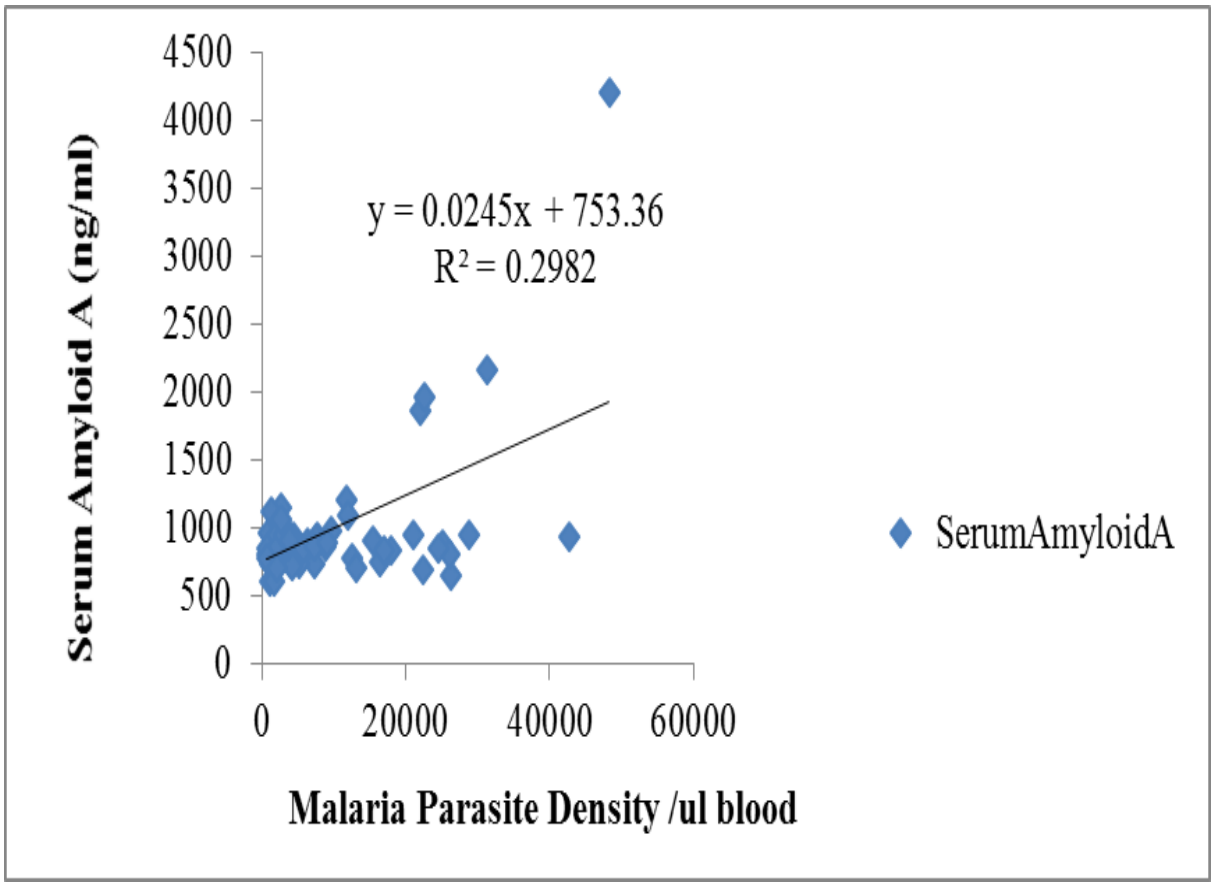


Figure 4.5: Effect of Malaria Parasite Density (MPD) on Serum Amyloid A of *P. falciparum* Infected Pregnant Women attending PHCs in Samaru Zaria

4.6.2 Variability in Serum amyloid A and C - reactive protein based on malaria parasite density

The secretions of both C - reactive protein (CRP) and Serum Amyloid A (SAA) increases with increase in malaria parasite density (Figure 4.6).

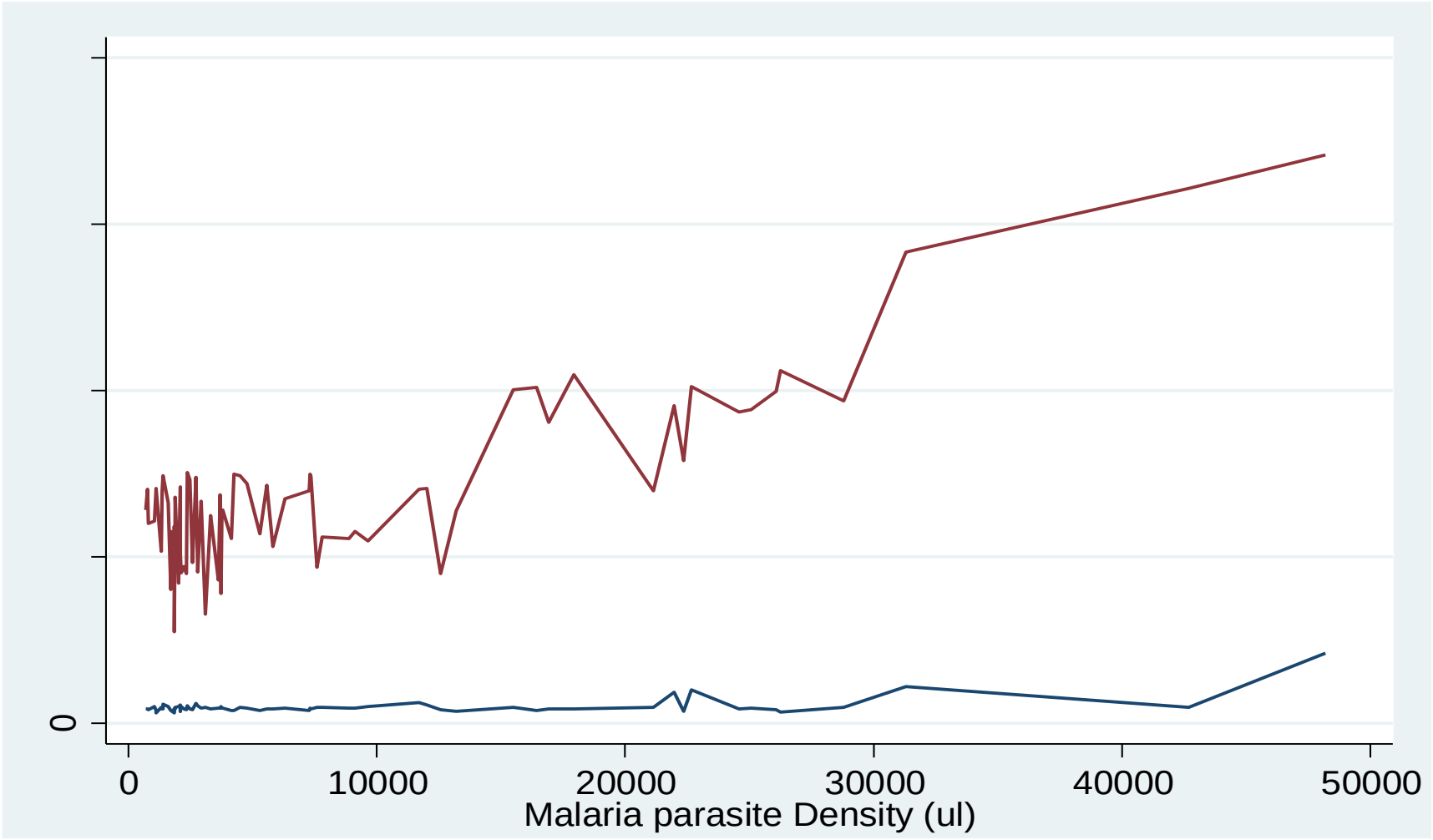


Figure 4.6: Variability in Serum amyloid A and C - reactive protein as function of Malaria (*P. falciparum*) parasite density in Infected Pregnant Women attending PHCs in Samaru, Zaria.

CHAPTER FIVE

5.0 DISCUSSIONS

The overall prevalence of *Plasmodiumfalciparum* infection in this study (Table: 4.1) was 13.7%. This rate is lower than that reported from Port Harcourt (26.0 %,), Abakaliki (29.0 %,), Okigwe (54.0%) and Oshogbo (72.0%) (Wogu*et al.,* 2013), Nwonwu*et al*., (2009), Nduka*et al.,* (2006) and Adefioye*et al*., (2007); respectively. Similarly, Ebako& Umberto (2012) carried out a decade long research work in sub-Saharan Africa between 2000 to 2011, reported that malaria prevalence in pregnant women attending antenatal clinics was 29.5% in East and Southern Africa and 35.1% in West and Central Africa. The variations in prevalence rate from different areas depend upon a number of factors which include the time of research, socio- demographic status of the pregnant women and effectiveness in implementation of the preventive measures to reduce maternal malaria in the study area. A trend of decrease in prevalence rate can be observed towards 2015. The intensity of campaign towards reduction of maternal mortality to the minimum by the year 2015, through the use of Intermittent Preventive Treatment of malaria in pregnancy (IPTp) and Insecticide Treated Nets (ITNs), might account for the lower prevalence rate observed in the study area compared to the previous ones.

Malaria prevalence was strongly associated with socio-demographic status (SDS), the findings in this work are consistent with other previous studies carried out in Sub-Saharan Africa in which measures of SDS such as age, education, occupation, housing type, including the uptake of intervention plays crucial roles Sonko*et al.,* (2014). The findings of Tusting*et al*., (2013) is also consistent with this study (Table: 4.2). However, these findings differ fromSoni*et al.,* (2008), who found no association between malaria and SDS. In

addition, the findings of this study are in contrast to a study of Onwujekwe*et al.,* (2009) in Southeastern Nigeria based on self-reported malaria or fever, which found more malaria among better off SDS and Urban dwellers compared to the poor. Even though majority resided off- campus, the environmental hygiene of the study area plays an important role, leading to fewer incidences of malaria infections. This agrees with the work of Adefioye*et al*., 2007; which stated that poor sanitation increases the risk of malaria infections.

Malaria (*P. falciparum*) parasitemia in relation to age showed that older pregnant women had reduced malaria prevalence rate suggesting higher immunity. This finding (Table: 4.2) is similar to reports by other researches carried out in Nigeria, (Nduka*et al.,* 2006, Nnaji*et al*., 2008). The different malaria prevalence rate observed among these age groups could be attributed to the level of acquired immunity that increases with age, which may also be associated with protection from malaria infection. The high prevalence rate and higher mean parasite (*P. falciparum*) density at early age correlated with the work of Adefioye*et al.,* (2007).

The use of ITNs, IPTp and ITN+ IPTp were found to be highly effective in reducing the episodes of malaria among the pregnant women. However availability, affordability and awareness of these measures plays a crucial role in the prevention of malaria infection. ITNs reduces human-vector contact and the use benefits the pregnant woman as well as her family by reducing malaria related illness and death. Unfortunately, majority of the pregnant women have never used the ITNs at all (Figure: 4.1), it could be due to reasons earlier stated. This research finding agrees with the work of Wagbatsoma and Omoike (2008), who gave reason that the cost of combining ITNs with IPTpmay only be affordable to the affluent. Unlike the earlier prophylactic anti-malaria drugs that were given at less than

therapeutic dose and at regular interval which could encourage parasite resistance; IPTp using sulfadoxime-pyrimethamine (SP) employs a therapeutic dose regimen with only two doses at monthly interval during the course of pregnancy which effectively reduces parasite load. Majority of the pregnant mothers 376 (68.7%) received IPTp during clinic visit, still lower than 80% target by 2015, according to RBM report, but with increase as compared to WMR of 2014; that 20m of 35m (57%) pregnant women in sub-Saharan Africa did not receive single dose of IPTp in 2013.

Younger women (Primigravidae and Secondigravidae), particularly adolescents, are at higher risk of malaria infection than older women and this is independent of parity (Hamer*et al*., 2009). The high prevalence rate and high mean parasite(*P. falciparum)* density obtained within the first and second trimesters in this study (Table: 4.3), agreed with those of other studies which observed peak prevalence in weeks 10-20 of pregnancy (Zhou *et al.*,2001). This may be attributed to the expression of adherent proteins on the surface of infected red blood cells (IRBCs), enabling the IRBCs to adhere to micro-vascular capillaries of vital organs causing severe pathological conditions (Wogu *et al.,* 2013). Also, parity played a role in the prevalence rate as well as the mean parasite density. Primigravidae had a higher infection rate then multigravida. The high prevalence rate was in accordance with report of Mariell*et al.* (2003), in pregnant women in Gabon and was higher in primigravidae than multigravida Adefiye*et al.* (2007). *Plasmodium falciparum*infection was more common in primigravidae than in multigravida and was associated with mild anemia and an increased risk of Low Birth Weight (Nosten*et al*., 1999). Primigravidae are known to have increased severity of malaria infection and worst pregnancy outcome than multigravida in malaria endemic area (Rogerson*et al.,* 2007, Jennifer *et al.*, 2012). Even in

unstable transmission area, malaria can cause maternal anemia more in primigravidae than in multigravida and for *falciparum* infection more than for *vivax* infection (Ebako and Umberto, 2013). Nevertheless, severe anemia is less common in these settings.

The effect of *falciparum* infection on the pregnant woman is mostly dependent on pre-pregnancy immunity. This acquired anti-malaria immunity depends on intensity of transmission and the number of previous pregnancies among others. In areas of stable malaria transmission, the ill health effect is particularly apparent in the primigravidae than multigravida (WHO, 2014). Some studies correlates with the current research that higher prevalence rate and the density of parasitemia is reported to be highest in the first trimester and primigravida (Table: 4.3) which could be at maximum risk in highly endemic area (Wagbatsoma and Omoike, 2008). Pregnant women have higher risk of malaria compared to non-pregnant women (Adefioye*et al.*, 2007; Ebako and Umberto, 2013), due to low maternal age, low parity and low gestational age. The present study did not agree with their finding. Instead, the malaria attack is less during pregnancy (Figure: 4.2), since the maternal risk factors are not the same. The child spacing practiced in the community could help in boosting and stabilizing the immunity of the pregnant mothers, as frequent pregnancy affects the immune system negatively hence increases the risk of malaria infection(Figure: 4.3).

The indices of Iron status, particularly hemoglobin, serum ferritin and soluble transferrin receptors (sTfR) concentration relate to changes in the acute phase proteins during inflammation. The changes in the concentration of serum acute phase proteins and markers of iron status during treatment for infection are used to demonstrate inter-relationships between the indicators. Malaria produces an acute phase response and relationships between

Acute Phase Proteins(APP) and indices of iron status indicates an influence of inflammation in both symptomatic and asymptomatic malaria, except when the parasitaemia is less than 1000/ul of blood when ferritin appears to be unaffected (Northrop-clewes, 2008).

Anaemia and iron deficiency were determined by use of haemoglobin concentration and serum iron levels respectively. The acute phase response to infection alters the plasma concentrations of most biochemical measures of iron status, rendering assessment of status difficult. Soluble transferrin receptors (sTfR) may be an exception as is the case of malaria infection with iron deficiency anaemia (Richard *et al.*, 2000). Pregnant women with malaria and concomitant iron deficiency anaemia had significantly high sTfR levels as in this study. This was explained by Saboor (2011), that 80% of sTfR relate to the erythroid precursors. Decreased availability of iron to the erythroid precursors-causes increased level of sTfR. Hence, in iron deficient individuals sTfR levels are significantly increased. Some other research findings are variance, Menendez *et al*., (2010) and Verhoef*et al*., (2010) who found increased sTfR level in all patients with malaria.

The higher levels of serum ferritin among pregnant mothers with parasitemia, has also agreed with the work of Odunukwe*et al*., (2007) who reported that serum ferritin is elevated in symptomatic malaria infection. As a known acute phase reactant protein, its concentration in the serum is known to increase during acute inflammatory reactions. *Plasmodium falciparum* patient's serum ferritin was significantly higher than those infected with *Plasmodium Malariae* (Odunukwe*et al.*, 2007). This could be explained by the fact that haemolysis is most severe with *Plasmodium falciparum* which invades red cells of all ages while *Plasmodium malaria*e invades mainly normoblast (Strickland, 1986).

The linear correlation between serum ferritin and the malaria parasite density especially *Plasmodiumfalciparum*, throw more light on the actual sources of ferritin in malaria infection. This study exposes the fallacy of measuring the serum ferritin level in a malaria-endemic area without first determining the malaria parasitemia status.

Although the zinc level was significantly lower, the levels of copper and C-reactive protein were significantly higher in pregnant women with malaria. There was a significant inverse correlation between zinc and C-reactive protein and significant positive correlation between copper and C-reactive protein. This studyis in concordance with the work of Sa'ad*et al.*, (2012) who concluded that change in zinc and copper may play a role in pathogenesis of *Plasmodiumfalciparum* malaria.

The reduction of serum trace elements concentration in infected population justifies works of some authors who associated malaria infestation to micronutrient concentration (Muller and Kappas, 2007). In their opinions, variability concentration of these nutrients in population suffering from malaria is assigned to *plasmodium* infection (Wander *et al.*, 2009). Iron as an essential element of hemoglobin which transports and stock oxygen in organism, playing a function in oxidative metabolism, cellular proliferation and other physiological process (Oppenheirmer, 2001). This nutrient is important for all pathogenic organism in free state, it may encourage their virulence.

Infection in organism generate reactive oxidant factors which activate a nucleus factor (NF-KB), which regulates gene-expression in immune response against pathogens agents (Owuor and Kong, 2002). Zinc protects cells against membrane peroxidation, by reducing about 50% activation of this nuclear factor (UNESCO, 2003). Zinc and copper co-factors to the functionality of superoxide dismutase; and antioxidant such as vitamin A, C and E. thus,

these nutrients (Zn and Cu) might partly be used by *plasmodium* to fight the oxidant flow generated in course of infection; that would explain their reduction in the infected patients as it has been obtained in the present study. The result is also in conformity with those of Shankar (2000), where during the acute-phase response, zinc is redistributed from plasma to lymphocytes and to the liver, causing decreased zinc plasma concentration and a micro biostatic environment (Beisel*et al*., 1995).

In other ways, zinc participate to the restoration of immune function; its reduction would encourage the virulence of plasmodium because the parasite would find through the deficiency of subject's immunity a free way to express its metabolism and so its virulence (Prasad, 2000; Nussemblatt and Semba, 2002). Increasingly, the reduction of zinc concentration might unbalance the ratio Cu/Zn, so it would increase the oxidative stress in patients and would worsen their pathological state by the negative effects of oxidants reactive factors (Mezzetti*et al*., 1998). The cohabitation of parasite manifestation and nutritional deficiency of trace elements creates damage in the health of infected population (Melaine*et al.*, 2010).

Platelets are playing a fundamental function by converting fibrinogen into fibrin for blood coagulations (Melaine*et al*., 2010); their elimination in course of malaria would facilitate with the blood flow, the circulation and propagation of plasmodium. This explains the decrease in concentration of platelets in malaria infected patients, as obtained by this study.

None of the pregnant women had gross abnormality of biochemical and hematological indices. There was a significant correlation between the parasite count and markers of inflammation (C-reactive protein and serum Amyloid A). This agree with work of Gillespie *et al*., 1991, Yapi*etal*., 2010 and Sa'ad*et al.*, 2012. All believed that measurement of these

acute phase reactants may prove valuable in assessing the severity of *Plasmodium falciparum* malaria, and in following the response to antimalarial treatment such measurement have physiological relevance as they are an indirect measure of inflammatory mediators thought to be important in the pathogenesis of the disease. The C-reactive protein (CRP) is secreted in order to fight against *plasmodium falciparum* invasion (Volankis, 2001). It binds damaged host cells including erythrocyte infected by *plasmodium falciparum*, resulting in their clearance by both humoral and cellular immune mechanism (Ansar*et al.,* 2006). Moreover, Pannen and Robotham, (2005) suggested that measurement of acute phase proteins can be of diagnostic or prognostic value under certain clinical conditions. But here it seems difficult to propose useful prognostic or diagnostic markers considering their sensitivity without malaria in study areas as in the case of malaria negative patients. While Hurt *et al*. (2004), have proposed CRP as a tool in malaria epidemiology studies to measure fevers. Interestingly, it was detected that only serum Amyloid A (SAA) binds with hemozoin, but not CRP. The absence of lipid-binding domain in CRP could explain its failure to interact with hematozoin (Fikregabrail*et al.,* 2011). Serum Amyloid A (SAA) is an acute phase protein whose level increases during infection, the result of this work showed that SAA level increases in the serum of malaria patients as stated by Urien-Shoral*et al.,* (2000).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of Malaria in Pregnancy (MiP) in the study area was low, this could be as a result of high compliance to IPTp that was mostly used at the second trimester by the multigravida pregnant mothers. The Indices of iron status indicated higher level in those pregnant mothers with combined malaria infection and iron deficiency anemia, which is very critical in pregnancy. The nutritional status of both pregnant women with parasitemia and those without parasitemia did not show any significant difference, except alteration in Zn/Cu ratio among the infected population.

It is expected that the use of sequential measurement of inflammation biomarkers, especially C-reactive protein and serum Amyloid A will improve the diagnosis of subclinical malaria.

6.2 Recommendations

i. Insecticide treated mosquito nets (ITNs) should be readily available and affordable if possible free to all pregnant mothers not only on clinic visit but to be given at homes.

ii. There's need to intensify campaign on the importance of child spacing as the gap between pregnancies plays a substantial role in decreasing maternal mortality among other factors.

iii. It's important to include Ferritin investigations during pregnancy as it detects iron deficiency which is very critical to pregnant mothers

iv. Among the inflammatory proteins especially serum amyloid A can be used to identify the intensity of malaria infection.

6.3Contribution to Knowledge

This study has established that**:**

(i) By assessment the target coverage of 90% IPTp and 80% ITNs implementation nationwide by 2015 (RBM, 2000), has not being achieved at least in this environment; instead 68.7% IPTp and 32.0% was obtained based on this research.

(ii) The use of some Inflammation biomarkers (CRP, SAA) has proved valuable in the diagnosis and monitoring of malaria infection provided some variables are combined in an index.

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APPENDICES

APPENDIX I

Assessment of Nutritional Status and Acute Phase Protein Levels among Pregnant Women with Malaria Questionnaire

1. PHC ……
2. S/NO……
3. Age (years): 15-19( ), 20-24( ), 25-29( ), 30-34( ), 35-39( ), >40( ).
4. Educational background; Non-Formal/Education( ), primary( ), secondary( ), Tertiary ( )
5. Occupation of Housewife; Civil servant ( ), Full H/wife ( ), Petty trading ( ) Full time business ( )
6. Place of residence; in- campus ( ), off campus ( )
7. Gestation period; 1st trimester ( ), 2ndtrimester ( ) 3rd trimester ( )
8. Date of last delivery; 1year ( ), 2 years ( ), 3 years ( ), Above 4 ( )
9. Number of pregnancies; primigravid ( ) Multigravida ( )
10. At which stage do you mostly experience malarial attack?

    Pregnant ( ), Non- pregnant ( )

11. How many of the pregnancies ended in: miscarriage ( ), stillbirth ( )
12. Use of Net [ITNs]; always ( ), not always ( ), not at all ( )
13. Compliance with ITP_p ; yes( ), No( )
14. BMI…… Height……weight…….MUAC……

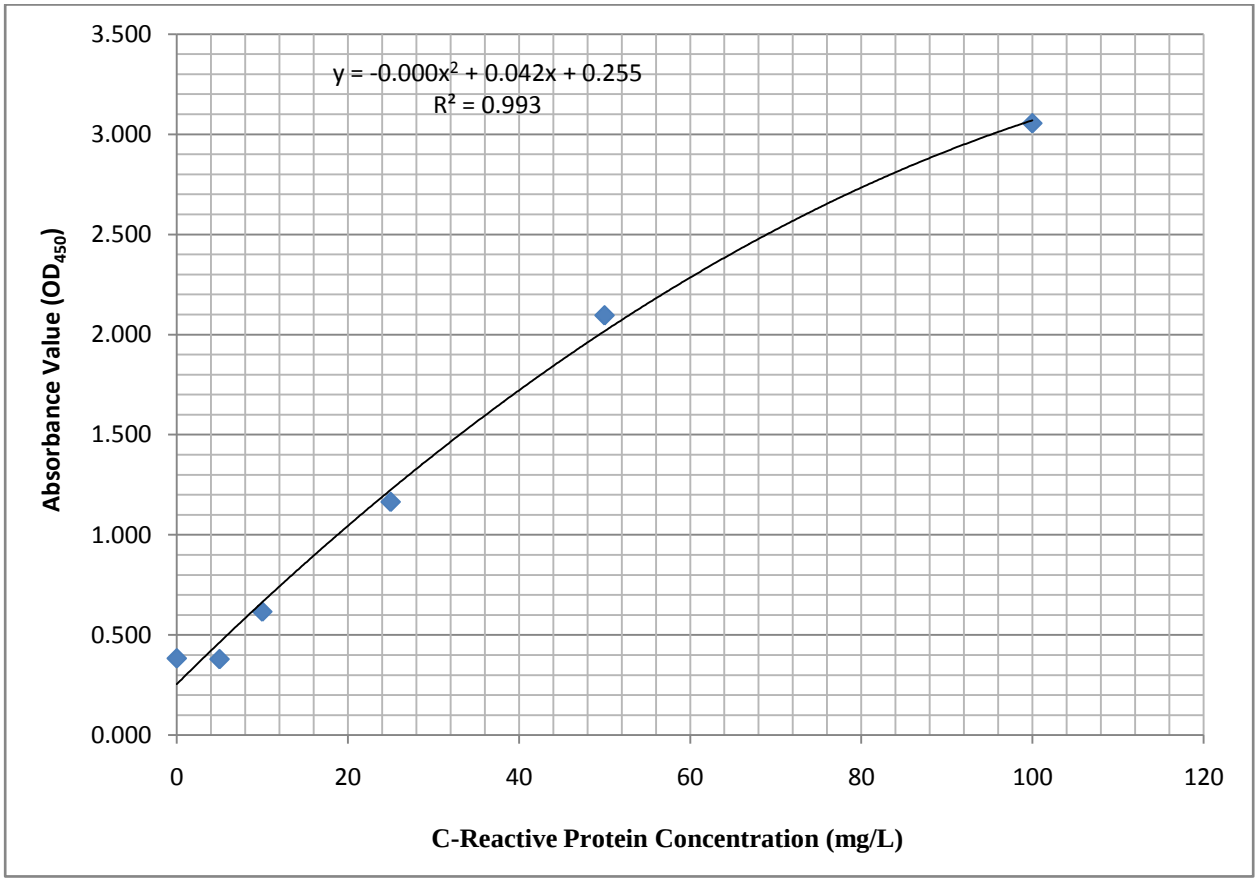
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APPENDIX II

C-REACTIVE PROTEIN RESULT INTERPRETATION

SN	STANDARDS CONCENTRATION	OPTICAL DENSITY
1	0	0.384
2	5	0.380
3	10	0.617
4	25	1.165
5	50	2.096
6	100	3.055



C-REACTIVE PROTEIN RESULT INTERPRETATION

Note: Sera sample were diluted 100 fold before assay hence, estimated concentrations obtained must be multiplied by 100 to get the actual concentration in undiluted sera samples

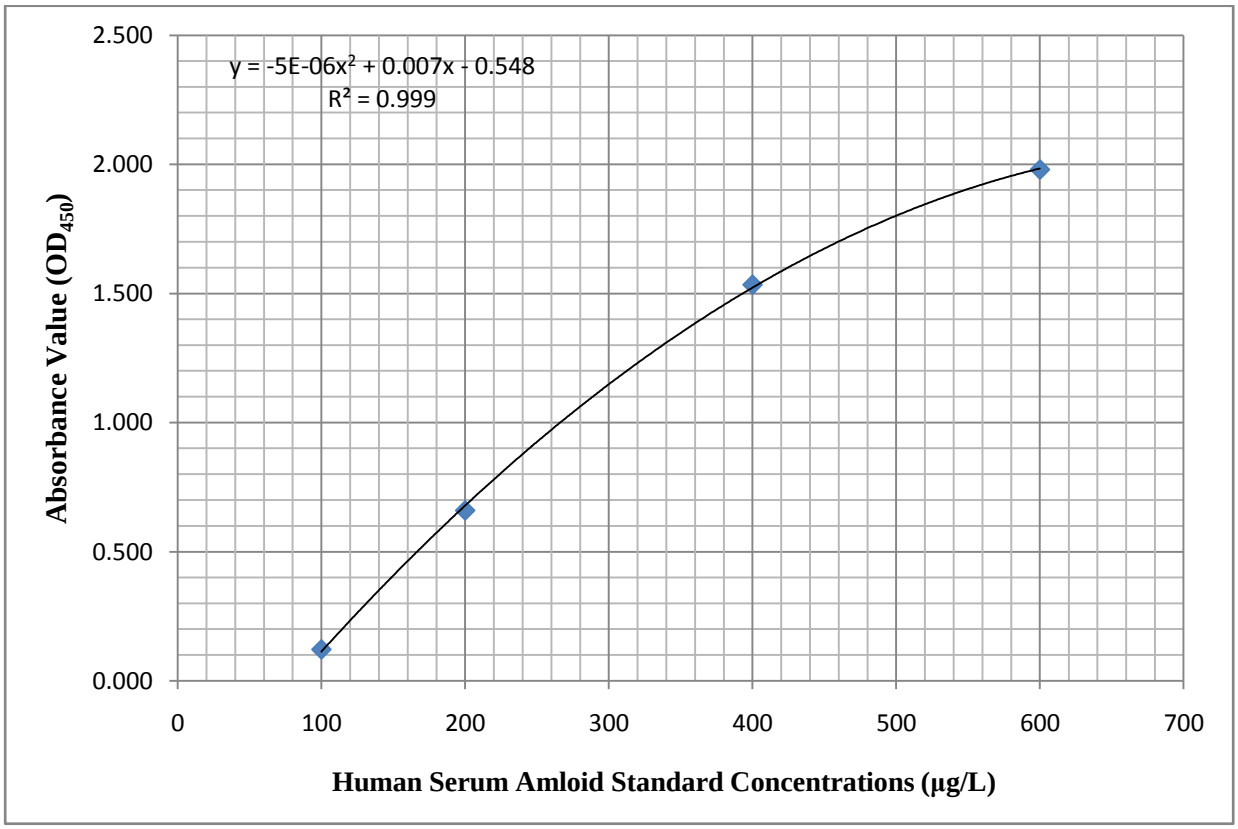
SN	SAMPLE	OPTICAL DENSITY	EST CONC. In diluted sera sample	Actual conc. In Sera Samples
1	Control level 1	0.191	3.96	395.55
2	Control level 2	0.237	4.46	446.44
3	SAMPLE 1	1.302	25.55	2554.88
4	SAMPLE 2	4.000	158.78	15878.03
5	SAMPLE 3	3.739	140.89	14089.12
6	SAMPLE 4	2.726	81.61	8160.55
7	SAMPLE 5	2.687	79.65	7964.56
8	SAMPLE 6	2.486	69.92	6992.37
9	SAMPLE 7	2.589	74.83	7482.62
10	SAMPLE 8	0.040	2.52	251.88
11	SAMPLE 9	2.652	77.91	7790.70
12	SAMPLE 10	2.589	74.83	7482.62
13	SAMPLE 11	0.305	5.28	527.76
14	SAMPLE 12	2.369	64.56	6455.73
15	SAMPLE 13	2.934	92.46	9246.23
16	SAMPLE 14	2.534	72.19	7218.76
17	SAMPLE 15	2.009	49.40	4939.50
18	SAMPLE 16	3.084	100.71	10071.39
19	SAMPLE 17	2.940	92.79	9278.56
20	SAMPLE 18	1.246	24.00	2399.60
21	SAMPLE 19	0.604	9.72	971.62
22	SAMPLE 20	3.221	108.56	10855.94
23	SAMPLE 21	2.901	90.69	9069.44
24	SAMPLE 22	3.084	100.71	10071.39
25	SAMPLE 23	2.982	95.06	9506.43
26	SAMPLE 24	3.084	100.71	10071.39
27	SAMPLE 25	3.147	104.29	10428.50
28	SAMPLE 26	3.428	120.97	12097.35
29	SAMPLE 27	3.030	97.70	9770.25
30	SAMPLE 28	2.740	82.31	8231.49
31	SAMPLE 29	1.998	48.96	4896.38
32	SAMPLE 30	3.020	97.15	9714.99
33	SAMPLE 31	3.211	107.98	10797.68
34	SAMPLE 32	2.536	72.28	7228.28
35	SAMPLE 33	2.429	67.28	6728.24
36	SAMPLE 34	3.172	105.72	10571.95
37	SAMPLE 35	3.491	124.89	12488.55
38	SAMPLE 36	3.491	124.89	12488.55
39	SAMPLE 37	3.417	120.30	12029.69
40	SAMPLE 38	3.355	116.52	11651.87

41	SAMPLE 39	1.388	28.03	2802.96
42	SAMPLE 40	2.139	54.64	5463.52
43	SAMPLE 41	3.114	102.41	10240.66
44	SAMPLE 42	2.990	95.50	9550.15
45	SAMPLE 43	2.949	93.27	9327.15
46	SAMPLE 44	1.435	29.43	2943.46
47	SAMPLE 45	3.012	96.71	9670.90
48	SAMPLE 46	3.144	104.11	10411.36
49	SAMPLE 47	3.114	102.41	10240.66
50	SAMPLE 48	1.738	39.33	3932.56
51	SAMPLE 49	3.086	100.83	10082.63
52	SAMPLE 50	3.251	110.32	11031.68
53	SAMPLE 51	2.605	75.60	7560.27
54	SAMPLE 52	3.130	103.32	10331.52
55	SAMPLE 53	3.033	97.87	9786.86
56	SAMPLE 54	3.033	97.87	9786.86
57	SAMPLE 55	3.258	110.73	11072.89
58	SAMPLE 56	1.052	19.00	1899.76
59	SAMPLE 57	3.063	99.54	9953.74
60	SAMPLE 58	3.168	105.49	10548.93
61	SAMPLE 59	3.258	110.73	11072.89
62	SAMPLE 60	1.444	29.71	2970.76
63	SAMPLE 61	3.446	122.08	12208.49
64	SAMPLE 62	3.536	127.72	12771.79
65	SAMPLE 63	3.814	145.92	14592.20
66	SAMPLE 64	3.057	99.20	9920.25
67	SAMPLE 65	3.057	99.20	9920.25
68	SAMPLE 66	2.892	90.22	9021.52
69	SAMPLE 67	3.141	103.94	10394.22
70	SAMPLE 68	4.000	158.78	15878.03
71	SAMPLE 69	3.290	112.62	11262.26
72	SAMPLE 70	2.463	68.85	6885.18
73	SAMPLE 71	3.529	127.28	12727.52
74	SAMPLE 72	1.834	42.76	4276.06
75	SAMPLE 73	2.987	95.34	9533.74
76	SAMPLE 74	3.247	110.08	11008.17
77	SAMPLE 75	3.209	107.86	10786.05
78	SAMPLE 76	3.543	128.16	12816.14
79	SAMPLE 77	3.542	128.10	12809.80
80	SAMPLE 78	2.907	91.01	9101.46
81	SAMPLE 79	3.130	103.32	10331.52
82	SAMPLE 80	3.260	110.85	11084.68
83	SAMPLE 81	3.825	146.67	14666.73
84	SAMPLE 82	3.660	135.69	13568.76
85	SAMPLE 83	3.314	114.05	11405.34
86	SAMPLE 84	3.260	110.85	11084.68
87	SAMPLE 85	3.452	122.46	12245.64
88	SAMPLE 86	3.260	110.85	11084.68
89	SAMPLE 87	3.660	135.69	13568.76

APPENDIX III

Human Serum Amyloid Interpretation

SN	STANDARDS CONCENTRATION	Optical Density	Mean (Optical Density)
1	600	1.796	1.980
2	600	2.163	
3	400	1.267	1.534
4	400	1.801	
5	200	0.439	0.66
6	200	0.881	
7	100	0.131	0.122
8	100	0.113	
9	50	0.171	0.159
10	50	0.147	



Human Serum Amyloid Interpretation

Note: Sera sample were diluted 5 fold (i.e. 10µl of sample in 40µl of sample diluent) before assay hence, estimated concentrations obtained must be multiplied by 5 to get the actual concentration in undiluted sera samples

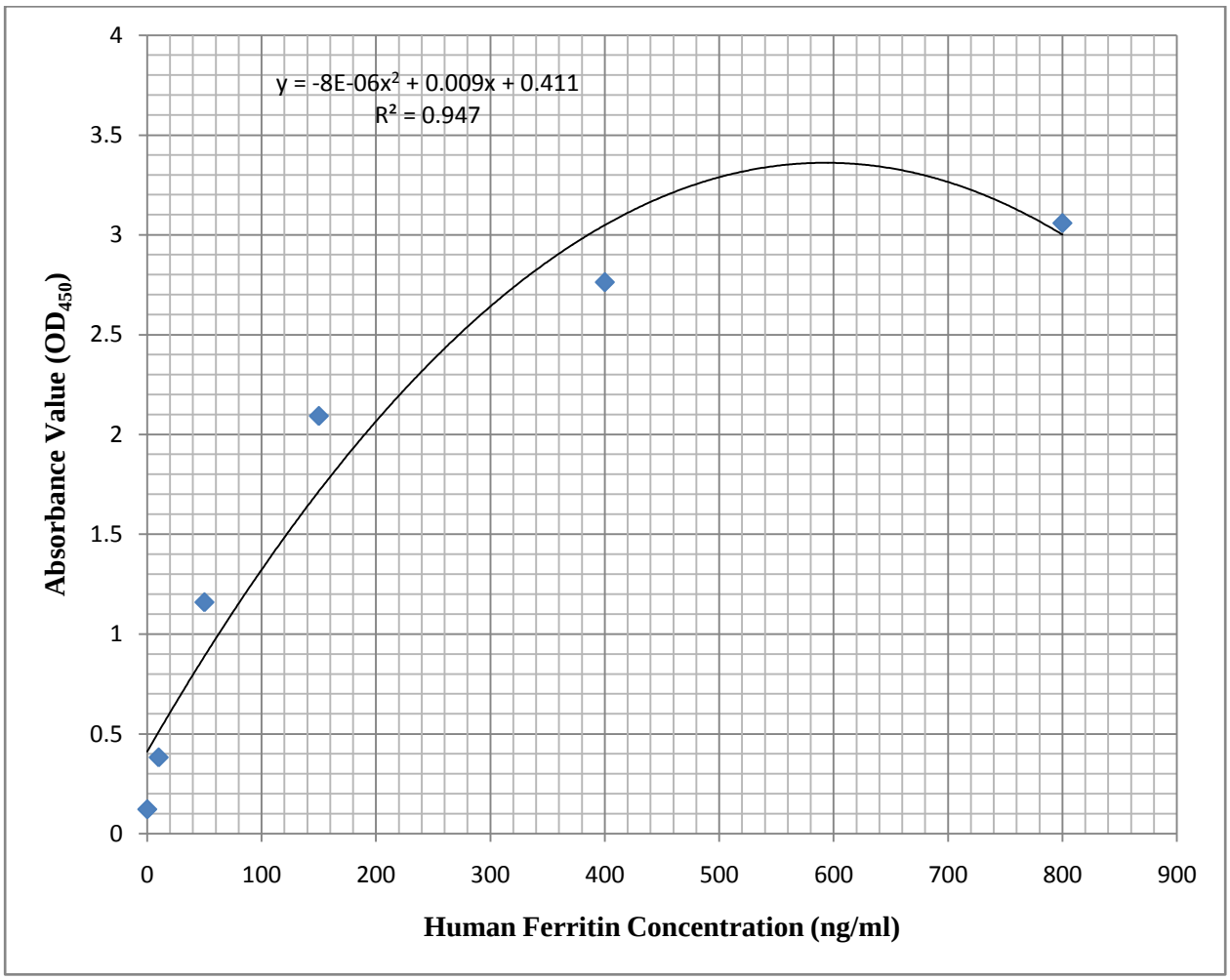
SN	SAMPLE	OPTICAL DENSITY	EST CONC. In diluted sera sample	Actual conc. In Sera Samples
1	SAMPLE 1	0.147	45.070	225.350
2	SAMPLE 2	0.230	77.583	387.914
3	SAMPLE 3	0.000	-22.234	-111.170
4	SAMPLE 4	0.019	-12.774	-63.871
5	SAMPLE 5	1.946	580.264	2901.319
6	SAMPLE 6	0.033	-5.955	-29.773
7	SAMPLE 7	0.042	-1.637	-8.186
8	SAMPLE 8	0.098	24.092	120.459
9	SAMPLE 9	0.105	27.174	135.871
10	SAMPLE 10	0.117	32.391	161.956
11	SAMPLE 11	0.252	85.606	428.029
12	SAMPLE 12	0.145	44.241	221.204
13	SAMPLE 13	2.655	1209.015	6045.077
14	SAMPLE 14	0.379	127.575	637.877
15	SAMPLE 15	0.319	108.628	543.139
16	SAMPLE 16	0.230	77.583	387.914
17	SAMPLE 17	0.202	67.020	335.101
18	SAMPLE 18	0.131	38.372	191.860
19	SAMPLE 19	0.132	38.795	193.974
20	SAMPLE 20	0.226	76.098	380.491
21	SAMPLE 21	0.146	44.656	223.278
22	SAMPLE 22	0.237	80.161	400.807
23	SAMPLE 23	0.056	4.977	24.885
24	SAMPLE 24	0.234	79.059	395.296
25	SAMPLE 25	0.164	52.030	260.150
26	SAMPLE 26	0.106	27.612	138.061
27	SAMPLE 27	0.296	100.956	504.782
28	SAMPLE 28	0.000	-22.234	-111.170
29	SAMPLE 29	0.000	-22.234	-111.170
30	SAMPLE 30	0.214	71.596	357.980
31	SAMPLE 31	0.073	12.844	64.219
32	SAMPLE 32	0.124	35.396	176.979
33	SAMPLE 33	0.098	24.092	120.459
34	SAMPLE 34	0.313	106.649	533.246
35	SAMPLE 35	0.303	103.316	516.581
36	SAMPLE 36	0.198	65.478	327.392
37	SAMPLE 37	0.180	58.436	292.179
38	SAMPLE 38	0.413	137.674	688.369
39	SAMPLE 39	0.315	107.310	536.552
40	SAMPLE 40	0.274	93.395	466.973

41	SAMPLE 41	0.125	35.823	179.113
42	SAMPLE 42	0.272	92.696	463.480
43	SAMPLE 43	0.163	51.625	258.125
44	SAMPLE 44	0.050	2.157	10.787
45	SAMPLE 45	0.268	91.293	456.466
46	SAMPLE 46	0.216	72.351	361.757
47	SAMPLE 47	0.372	125.441	627.204
48	SAMPLE 48	1.305	334.940	1674.700
49	SAMPLE 49	0.293	99.938	499.692
50	SAMPLE 50	0.200	66.250	331.252
51	SAMPLE 51	0.418	139.122	695.611
52	SAMPLE 52	0.275	93.743	468.716
53	SAMPLE 53	0.184	60.016	300.078
54	SAMPLE 54	0.263	89.529	447.645
55	SAMPLE 55	0.147	45.070	225.350
56	SAMPLE 56	0.167	53.242	266.208
57	SAMPLE 57	0.154	47.955	239.776
58	SAMPLE 58	0.202	67.020	335.101
59	SAMPLE 59	0.275	93.743	468.716
60	SAMPLE 60	0.304	103.651	518.257
61	SAMPLE 61	0.240	81.259	406.296
62	SAMPLE 62	0.258	87.753	438.765
63	SAMPLE 63	0.232	78.322	391.610
64	SAMPLE 64	0.264	89.883	449.414
65	SAMPLE 65	0.295	100.618	503.088
66	SAMPLE 66	0.186	60.802	304.012
67	SAMPLE 67	0.083	17.388	86.942
68	SAMPLE 68	0.152	47.134	235.669
69	SAMPLE 69	0.271	92.346	461.730
70	SAMPLE 70	0.141	42.575	212.876
71	SAMPLE 71	0.336	114.149	570.745
72	SAMPLE 72	0.121	34.112	170.558
73	SAMPLE 73	0.269	91.645	458.223
74	SAMPLE 74	0.276	94.091	470.457
75	SAMPLE 75	0.259	88.109	440.546
76	SAMPLE 76	0.290	98.916	494.581
77	SAMPLE 77	1.613	427.296	2136.478
78	SAMPLE 78	0.217	72.728	363.642
79	SAMPLE 79	0.073	12.844	64.219
80	SAMPLE 80	0.084	17.839	89.197
81	SAMPLE 81	0.097	23.649	118.246
82	SAMPLE 82	0.630	193.268	966.342
83	SAMPLE 83	0.304	103.651	518.257
84	SAMPLE 84	0.265	90.236	451.181
85	SAMPLE 85	0.142	42.992	214.962

APPENDIX IV

Human Serum Ferritin Interpretation

SN	STANDARDS CONCENTRATION	OPTICAL DENSITY
1	0	0.122
2	10	0.382
3	50	1.159
4	150	2.092
5	400	2.762
6	800	3.058



HUMAN SERUM FERRITIN INTERPRETATION

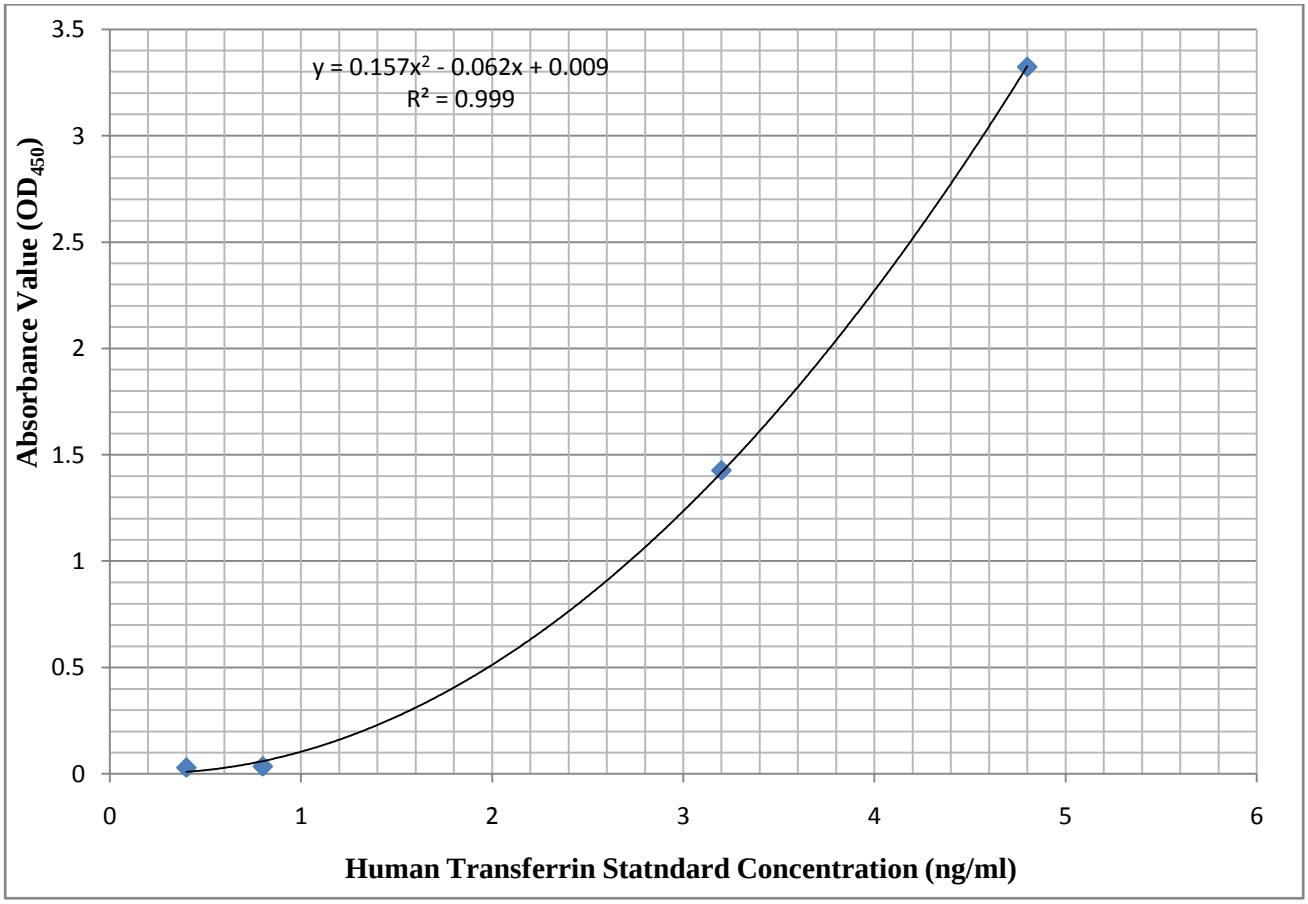
SN	SAMPLE	OPTICAL DENSITY	EST CONC. In sera sample
1	SAMPLE 1	0.501	12.844
2	SAMPLE 2	0.44	11.479
3	SAMPLE 3	0.475	12.283
4	SAMPLE 4	1.63	46.230
5	SAMPLE 5	2.818	429.636
6	SAMPLE 6	2.095	150.745
7	SAMPLE 7	0.226	4.793
8	SAMPLE 8	0.187	3.139
9	SAMPLE 9	0.691	16.397
10	SAMPLE 10	0.326	8.361
11	SAMPLE 11	0.399	10.455
12	SAMPLE 12	0.6	14.772
13	SAMPLE 13	0.182	2.914
14	SAMPLE 14	0.229	4.914
15	SAMPLE 15	0.143	1.069
16	SAMPLE 16	0.34	8.793
17	SAMPLE 17	0.423	11.066
18	SAMPLE 18	1.82	93.869
19	SAMPLE 19	2.243	191.210
20	SAMPLE 20	0.159	1.848
21	SAMPLE 21	1.146	51.877
22	SAMPLE 22	1.068	54.986
23	SAMPLE 23	0.324	8.299
24	SAMPLE 24	0.852	19.467
25	SAMPLE 25	1.04	24.465
26	SAMPLE 26	0.591	14.606
27	SAMPLE 27	0.216	4.384
28	SAMPLE 28	2.084	148.030
29	SAMPLE 29	0.467	12.104
30	SAMPLE 30	0.266	6.330
31	SAMPLE 31	1.221	48.948
32	SAMPLE 32	1.168	50.999
33	SAMPLE 33	1.246	48.033
34	SAMPLE 34	0.512	13.073
35	SAMPLE 35	0.335	8.640
36	SAMPLE 36	1.983	124.887
37	SAMPLE 37	1.33	38.529
38	SAMPLE 38	2.063	142.956
39	SAMPLE 39	0.961	22.091
40	SAMPLE 40	1.468	49.311
41	SAMPLE 41	0.726	17.022
42	SAMPLE 42	1.38	42.074
43	SAMPLE 43	3.037	739.175
44	SAMPLE 44	1.141	52.078
45	SAMPLE 45	0.926	21.177

46	SAMPLE 46	0.677	16.149
47	SAMPLE 47	0.831	19.026
48	SAMPLE 48	0.938	21.482
49	SAMPLE 49	0.188	3.183
50	SAMPLE 50	0.93	21.278
51	SAMPLE 51	0.577	14.346
52	SAMPLE 52	1.438	46.694
53	SAMPLE 53	0.416	10.891
54	SAMPLE 54	2.143	163.054
55	SAMPLE 55	0.224	4.712
56	SAMPLE 56	0.515	13.134
57	SAMPLE 57	0.655	15.759
58	SAMPLE 58	1.666	47.980
59	SAMPLE 59	0.653	15.724
60	SAMPLE 60	1.177	50.643
61	SAMPLE 61	0.18	2.824
62	SAMPLE 62	0.705	16.645
63	SAMPLE 63	1.099	53.763
64	SAMPLE 64	0.777	17.962
65	SAMPLE 65	1.581	60.687
66	SAMPLE 66	1.186	50.289
67	SAMPLE 67	0.366	9.555
68	SAMPLE 68	0.477	12.327
69	SAMPLE 69	0.57	14.215
70	SAMPLE 70	1.499	52.187
71	SAMPLE 71	0.423	11.066
72	SAMPLE 72	0.496	12.738
73	SAMPLE 73	0.645	15.582
74	SAMPLE 74	1.383	42.299
75	SAMPLE 75	0.579	14.384
76	SAMPLE 76	0.574	14.290
77	SAMPLE 77	1.035	24.301
78	SAMPLE 78	0.387	10.136
79	SAMPLE 79	1.343	45.034
80	SAMPLE 80	0.924	21.127
81	SAMPLE 81	0.249	5.696
82	SAMPLE 82	0.792	18.249
83	SAMPLE 83	0.555	13.929
84	SAMPLE 84	0.741	17.294
85	SAMPLE 85	0.371	9.696
86	SAMPLE 86	0.839	19.192
87	SAMPLE 87	0.831	19.026
88	SAMPLE 88	0.374	9.780
89	SAMPLE 89	1.565	58.923

APPENDIX V

HUMAN SERUM TRANSFERRIN INTERPRETATION

SN	STANDARDS CONCENTRATION	Optical Density	Mean (Optical Density)
1	0.4	0.039	0.029
2	0.4	0.019	
3	0.8	0.024	0.0345
4	0.8	0.045	
5	1.6	0.169	1.616
6	1.6	3.063	
7	3.2	1.508	1.4265
8	3.2	1.345	
9	4.8	4.000	3.324
10	4.8	2.648	



Human Transferrin Interpretation

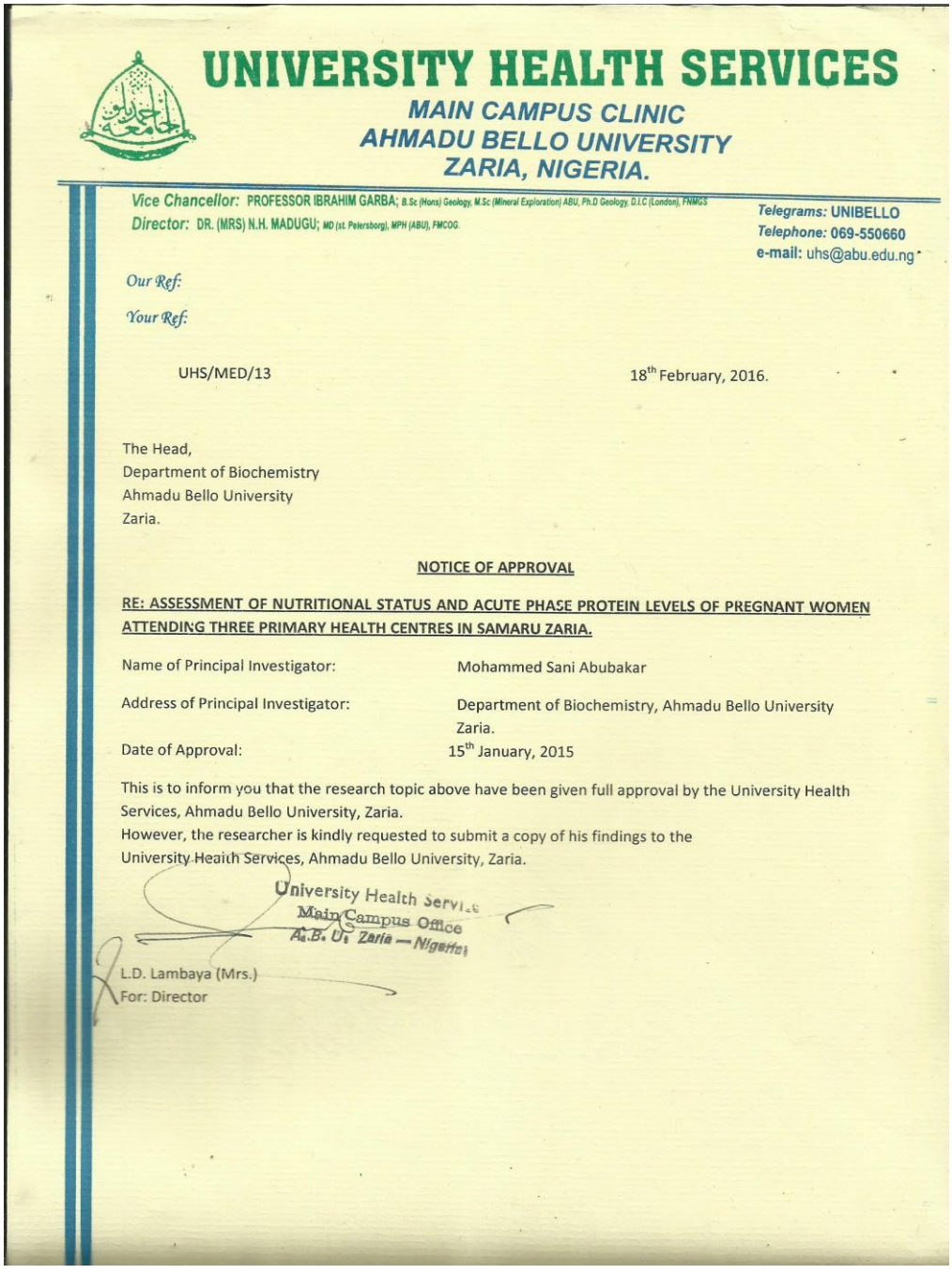
Note: Sera sample were diluted 5 fold (i.e. 10µl of sample in 40 µl of sample diluent) before assay hence, estimated concentrations obtained must be multiplied by 5 to get the actual concentration in undiluted sera samples

SN	SAMPLE	OPTICAL DENSITY	EST CONC. In diluted sera sample	Actual conc. In Sera Samples
1	SAMPLE 1	0.158	0.918	4.589
2	SAMPLE 2	0.057	0.688	3.441
3	SAMPLE 3	0	0.556	2.779
4	SAMPLE 4	0.081	0.743	3.716
5	SAMPLE 5	0.577	1.800	9.001
6	SAMPLE 6	0.086	0.755	3.774
7	SAMPLE 7	0.048	0.667	3.337
8	SAMPLE 8	0	0.556	2.779
9	SAMPLE 9	0	0.556	2.779
10	SAMPLE 10	0	0.556	2.779
11	SAMPLE 11	0.046	0.663	3.314
12	SAMPLE 12	0.022	0.607	3.035
13	SAMPLE 13	1.409	3.218	16.092
14	SAMPLE 14	0.289	1.206	6.029
15	SAMPLE 15	0.161	0.924	4.622
16	SAMPLE 16	0.077	0.734	3.671
17	SAMPLE 17	0.077	0.734	3.671
18	SAMPLE 18	0.9	2.403	12.017
19	SAMPLE 19	0.533	1.713	8.564
20	SAMPLE 20	0.3	1.229	6.147
21	SAMPLE 21	0.126	0.846	4.228
22	SAMPLE 22	0.013	0.586	2.930
23	SAMPLE 23	0.175	0.956	4.779
24	SAMPLE 24	0.045	0.660	3.302
25	SAMPLE 25	0.193	0.996	4.979
26	SAMPLE 26	0.183	0.974	4.868
27	SAMPLE 27	0.104	0.796	3.979
28	SAMPLE 28	0.109	0.807	4.036
29	SAMPLE 29	0.129	0.852	4.262
30	SAMPLE 30	0.114	0.819	4.093
31	SAMPLE 31	0.234	1.086	5.431
32	SAMPLE 32	0.112	0.814	4.070
33	SAMPLE 33	0.216	1.047	5.233
34	SAMPLE 34	0.218	1.051	5.255
35	SAMPLE 35	0.224	1.064	5.321
36	SAMPLE 36	0.066	0.709	3.544
37	SAMPLE 37	0.109	0.807	4.036
38	SAMPLE 38	0.354	1.345	6.723
39	SAMPLE 39	0.229	1.075	5.376
40	SAMPLE 40	0.268	1.160	5.802
41	SAMPLE 41	0.165	0.933	4.667

42	SAMPLE 42	0.19	0.989	4.945
43	SAMPLE 43	0.313	1.257	6.286
44	SAMPLE 44	0.131	0.857	4.285
45	SAMPLE 45	0.246	1.112	5.562
46	SAMPLE 46	0.087	0.757	3.785
47	SAMPLE 47	0.326	1.285	6.425
48	SAMPLE 48	0.618	1.880	9.402
49	SAMPLE 49	0.253	1.128	5.638
50	SAMPLE 50	0.214	1.042	5.211
51	SAMPLE 51	0.194	0.998	4.990
52	SAMPLE 52	0.189	0.987	4.934
53	SAMPLE 53	0.131	0.857	4.285
54	SAMPLE 54	0.0275	0.620	3.099
55	SAMPLE 55	0.204	1.020	5.101
56	SAMPLE 56	0.041	0.651	3.256
57	SAMPLE 57	0.12	0.832	4.161
58	SAMPLE 58	0.103	0.794	3.968
59	SAMPLE 59	0.306	1.242	6.211
60	SAMPLE 60	0.081	0.743	3.716
61	SAMPLE 61	0.215	1.044	5.222
62	SAMPLE 62	0.257	1.136	5.682
63	SAMPLE 63	0.222	1.060	5.299
64	SAMPLE 64	0.176	0.958	4.790
65	SAMPLE 65	0.172	0.949	4.745
66	SAMPLE 66	0.304	1.238	6.190
67	SAMPLE 67	0.312	1.255	6.276
68	SAMPLE 68	0.13	0.855	4.274
69	SAMPLE 69	0.223	1.062	5.310
70	SAMPLE 70	0.273	1.171	5.856
71	SAMPLE 71	0.314	1.259	6.297
72	SAMPLE 72	0.146	0.891	4.454
73	SAMPLE 73	0.237	1.093	5.464
74	SAMPLE 74	0.277	1.180	5.899
75	SAMPLE 75	0.216	1.047	5.233
76	SAMPLE 76	0.198	1.007	5.034
77	SAMPLE 77	0.671	1.983	9.913
78	SAMPLE 78	0.096	0.778	3.888
79	SAMPLE 79	0.287	1.201	6.007
80	SAMPLE 80	0.103	0.794	3.968
81	SAMPLE 81	0.074	0.727	3.636
82	SAMPLE 82	0.11	0.809	4.047
83	SAMPLE 83	0.258	1.139	5.693
84	SAMPLE 84	0.108	0.805	4.024
85	SAMPLE 85	0.175	0.956	4.779

APPENDIX VI

Ethical approval from University Medical Center ABU ZARIA



UNIVERSITY HEALTH SERVICES

MAIN CAMPUS CLINIC
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA.

Vice Chancellor: PROFESSOR IBRAHIM GARBA; B.Sc (Hons) Geology, M.Sc (Mineral Exploration) ABU, Ph.D Geology, D.I.C (London), FNMGS
Director: DR. (MRS) N.H. MADUGU; MD (st. Petersborg), MPH (ABU), FMCOG.

Telegrams: UNIBELLO
Telephone: 069-550660
e-mail: uhs@abu.edu.ng

Our Ref:
Your Ref:

UHS/MED/13 — 18th February, 2016.

The Head,
Department of Biochemistry
Ahmadu Bello University
Zaria.

NOTICE OF APPROVAL

RE: ASSESSMENT OF NUTRITIONAL STATUS AND ACUTE PHASE PROTEIN LEVELS OF PREGNANT WOMEN ATTENDING THREE PRIMARY HEALTH CENTRES IN SAMARU ZARIA.

Name of Principal Investigator:	Mohammed Sani Abubakar
Address of Principal Investigator:	Department of Biochemistry, Ahmadu Bello University Zaria.
Date of Approval:	15th January, 2015

This is to inform you that the research topic above have been given full approval by the University Health Services, Ahmadu Bello University, Zaria.
However, the researcher is kindly requested to submit a copy of his findings to the University Health Services, Ahmadu Bello University, Zaria.

University Health Service
Main Campus Office
A.B.U. Zaria — Nigeria

L.D. Lambaya (Mrs.)
For: Director

APPENDIX VII

Ethical approval from SabonGari

SABON GARI LOCAL GOVERNMENT

PRIMARY HEALTH CARE DEPARTMENT,
MEDICAL HEALTH UNIT

Local Government Secretariat, Dogarawa Headquarters, Kaduna State
P.M.B 1072

Our Ref: **SRLG/PHC/MED/001/VOL.I**
Your Ref:.....
To:.....

Date: 31st Jan 2015

The Head of Department,
Biochemistry Department,
A.B.U, Zaria.

Dear Sir,

ETHICAL APPROVAL

On behalf of Ethical Committee of the Sabon Gari Local Government of Kaduna State, your student 'Abubakar, M.S is allowed to carry out his MSC research work in Primary Health Care facility Samaru. He has completed his research successfully.

Bashir Ibrahim
Head of Medical Unit.

APPENDIX VIII

Informed Consent

ASSESSMENT OF NUTRITIONAL STATUS AND SOME ACUTE PHASE PROTEINS LEVELS OF PREGNANT WOMEN ATTENDING THREE PRIMARY HEALTH CARE CENTERS IN SAMARU ZARIA

This consent form gives information about the study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign your name or make your mark on this form. The researcher is from the Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria.

Purpose of the study
The purpose of this study is to assess the effect of malaria infection and acute phase protein production during pregnancy.

Study procedure

If you are eligible and decide to take part in the study, blood sample will be collected from you. Next a questionnaire will be given to you to fill.

Risk and/or discomfort
The only risk is during blood collection of which utmost care will be taken.

Benefits
This study is directly beneficial to you in the sense that it will ensure the development of an effective management of malaria in pregnancy and its complications.

Cost to you
There is no cost to you for being in this study.

Confidentiality
All records of your interviews will be kept in a safe place under lock and key. Your personal information may however be disclosed if required by law. Your records may be reviewed by authorised study staff and monitors

Consent statement for signature

I have read this entire consent form or the staff has read it to me, and has answered any questions.

I certify that I give my informed consent to take part in the survey Yes [ ] No [ ]

I agree to be contacted for a future study Yes [ ] No [ ]

_____ _____

Signature of interviewer Date

_____

Signature of the respondent