CYTOKINE PROFILE AND IRON STATUS OF PREGNANT WOMEN WITH MALARIA, INTESTINAL HELMINTHS AND HIV INFECTIONS IN IBADAN, NIGERIA

BY

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ABSTRACT

Malaria, helminthiasis and HIV are widespread infectious diseases in developing countries with heavy toll on pregnant women. Cases of co-infection exist due to similar environmental and human factors of transmission. The risk factors, control measures and pathology of these diseases on mother and child have been extensively studied but there is limited data on maternal iron status and cytokine profile, which are key determinants of a successful pregnancy. The aim of this study was to evaluate the interactions of these diseases on maternal iron status and peripheral blood cytokines.

Blood and stool samples were collected from apparently healthy 18-45 years old pregnant women recruited in different trimesters between August, 2011 and March, 2015. They were registered at the antenatal (n=490) and HIV clinics (n=217) of Adeoyo Maternity Hospital and Agbongbon Primary Healthcare Centre in Ibadan, Oyo State. Pretested questionnaires were administered for personal and socio-demographic details. Malaria parasitemia in Giemsa-stained thick blood films was examined microscopically. Stool samples were screened for helminths using Kato-Katz method. Absolute CD4 counts of HIV infected patients were determined by flow cytometry. Packed Cell Volume (PCV) was also determined. Ferritin and transferrin concentrations and cytokine levels (TNF-α, IFN-γ, IL-1α, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13 and IL-17) in serum were determined using ELISA. Iron level was determined by atomic absorption spectrophotometry. Data were analysed using descriptive statistics, logistic regression and Mann-Whitney U test at $\alpha_{0.05}$.

The mean age of the patients was 28.6±5.4 years old and majority (54.4%) were petty traders. Prevalence of infections were: 10.8% (malaria), 9.6% (helminths), 1.6% (malaria/helminths), 24% (malaria/HIV), 2.8% (helminths/HIV) and 0.5% (malaria/helminths/HIV). Co-infection of malaria and helminths had the lowest malaria parasite density (470 parasites/µL of blood) but the highest *Ascaris* egg count (1,959epg). The CD4 count of co-infection of HIV and malaria was <350 cells/mm^3. Residents of houses without toilet facilities (n=38) were 4.5 times more likely to be infected with helminths (OR=4.45, CI=1.71-11.55). There were 57.6% cases of low PCV (29.3% - 31.0%) and PCV values were significantly lower in those with malaria, HIV and co-infection of both relative to the uninfected. Concentrations of ferritin in the second trimester were significantly higher in single infection groups relative to the uninfected (19.50 ng/mL) with highest median values in malaria (121.07 ng/mL). In

second and third trimesters, median transferrin levels were significantly reduced in HIV (2,689.84 μg/mL; 2,528.72 μg/mL, respectively) and its co-infection with malaria (2,177.33 μg/mL; 2,865.75 μg/mL, respectively) relative to the uninfected (6,753.88 μg/mL; 6,542.88 μg/mL, respectively). Significant elevation occurred in IFN-γ in those with *Plasmodium* only relative to those with *Plasmodium* and helminth co-infection. In second and third trimesters, co-infection of malaria and HIV showed significant elevations in their cytokine profiles relative to their occurrences as single infections. Median serum iron concentration was significantly lower in malaria (0.3 mg/L) compared with the uninfected (1.24 mg/L).

Malaria co-infection with HIV predisposes to haemolytic anaemia and distortions in blood cytokines. However, helminth protects in co-infection with malaria or HIV with no profound impairment of the cytokine profiles.

Keywords: Malaria-HIV-Helminths co-infection, Pregnant women, Cytokine profile, Epidemiology, Ferritin

Word Count: 499

DEDICATION

This work is dedicated to the Almighty God for His uncommon grace and mercy towards me.

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CERTIFICATION

This is to certify that this research work was carried out by Olawunmi Rashidat, **RABIU** under my supervision in the Department of Zoology, University of Ibadan, Ibadan.

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TABLE OF CONTENTS

Content		Page
Title Page		
Abstract		i
Dedication		iii
Acknowledgement		iv
Certification		vii
Table of Contents		viii
List of Tables		xiii
List of Figures		xvii
List of Abbreviations		xviii
CHAPTER 1	INTRODUCTION	1
1.1	Background	1
1.2	Statement of Problem	2
1.3	Justification for the Research	4
1.4	Aim of this study	6
CHAPTER 2	LITERATURE REVIEW	7
2.1	Epidemiology of Malaria	7
2.2	Epidemiology of Soil-Transmitted Helminths Infections	10
2.3	Epidemiology of Human Immunodeficiency Virus (HIV) Infection	15
2.4	Co-infection	16
2.4.1	Malaria and Helminth Co-infection	16
2.4.2	Malaria and HIV Co-infection	17
2.4.3	Helminth and HIV Co-infection	18
2.5	Anaemia, Iron status and pregnancy	19
2.5.1	Infection associated anaemia and changes in iron status in pregnancy	21
2.6	Role of immune system in sustenance of pregnancy	23
2.6.1	Changes in cytokine profile in pregnancy caused by single infections	26

Content			Page

	2.6.2	Cytokine profile in response to co-infection: beneficial or detrimental	29
CHAPTER 3		METHODOLOGY	31
3.1		Study Area	31
3.2		Study Design	32
	3.2.1	Sample Size	32
3.3		Enrolment/Selection of Study Participants	33
3.4		Sample Collection	33
	3.4.1	Administration of questionnaires	34
3.5		Malaria microscopy and estimation of parasite density	34
3.6		Stool preparation and helminth quantification	35
3.7		Grouping of participants	35
3.8		Absolute CD4 count estimation	36
3.9		HIV RNA quantification for viral load determination	36
3.10		Enzyme-linked immunosorbent assay (ELISA) for determination of concentrations of ferritin, transferrin, cytokines and viral load	36
3.11		Packed Cell Volume determination	37
3.12		Serum iron determination	37
3.13		Ethical Consideration	37
3.14		Data Analysis	38
CHAPTER 4		RESULTS	39
4.1		Patients' socio-demographic details	39
4.2		Pattern and status of infection	42
4.3		Risk factors for susceptibility to malaria parasitemia and helminth infections	52
4.4		Anaemia in relation to infection status	55

Content			**Page**
4.5		Iron status in relation to infection	60
	4.5.1	Changes in Serum Ferritin (SF) levels in the presence of infection	62
	4.5.2	Serum Iron (SI) levels and infections	69
	4.5.3	Serum Transferrin (STf) levels in relation to infection status	75
4.6		Cytokine profile modification in response to single and co-infections	80
	4.6.1	Pro-inflammatory cytokine (PIC) profile modification in response to *Plasmodium* infection and its co-infection with helminths or HIV	82
	4.6.2	Pro-inflammatory cytokine (PIC) profile modification in response to helminth infections and its co-infection with *Plasmodium* or HIV	86
	4.6.3	Pro-inflammatory cytokine (PIC) profile modification in response to HIV infection and its co-infection with *Plasmodium* or helminths	90
	4.6.4	Anti-inflammatory cytokine (AIC) profile modification in response to *Plasmodium* and its co-infection with helminths or HIV	94
	4.6.5	Anti-inflammatory cytokine (AIC) profile modification in response to helminth and its co-infection with *Plasmodium* or HIV	97
	4.6.6	Anti-inflammatory cytokine (AIC) profile modification in response to HIV infection and its co-infection with *Plasmodium* or helminths	100
4.7		Relationship in parasite density, anaemia, iron status and cytokines	103

Content			**Page**
CHAPTER 5		DISCUSSION	114
5.1		Patients' demographic details	114
5.2		Prevalence and pattern of infections	114
5.3		Risk factors for *Plasmodium* and helminth infections	117
5.4		Anaemia prevalence, iron status and associated risks in pregnancy	118
	5.4.1	Packed Cell Volume and Infections	118
	5.4.2	Serum ferritin and Infections	120
	5.4.3	Serum iron and Infections	122
	5.4.4	Serum transferrin and Infections	123
5.5		Cytokine profile changes in pregnancy	124
	5.5.1	Pro-inflammatory cytokines (PIC) in *Plasmodium* and its co-infection with helminths or HIV	124
	5.5.2	Pro-inflammatory cytokines (PIC) in helminth and its co-infection with *Plasmodium* or HIV	126
	5.5.3	Pro-inflammatory cytokines (PIC) in HIV and its co-infection with *Plasmodium* or helminths	127
	5.5.4	Anti-inflammatory cytokines (AIC) in *Plasmodium* and its co-infection with helminths or HIV	129
	5.5.5	Anti-inflammatory cytokines (AIC) in helminth and its co-infection with *Plasmodium* or HIV	130
	5.5.6	Anti-inflammatory cytokines (AIC) in HIV and its co-infection with *Plasmodium* or helminths	131
5.6		Correlation in cytokine profile and iron status indices	132
CHAPTER 6		CONCLUSIONS AND RECOMMENDATIONS	134
	6.1	Conclusions and Recommendations	134

Content **Page**

6.2	Limitations of the study	135
6.3	Contributions to Knowledge	135
	REFERENCES	137
	APPENDIX	163

LIST OF TABLES

Content **Page**

Table 2.1: Functions of cytokines 24

Table 4.1: Socio-demographic characteristics of recruited pregnant women 40

Table 4.2: Prevalence of infection in the study population. 43

Table 4.3: Geometric mean count of parasite density, cell count and viral load among the infected groups 44

Table 4.4: Prevalence of infections by age, trimester and gravidity 46

Table 4.5: Malaria parasite density in relation to age, trimester and gravidity 47

Table 4.6: *Ascaris* egg count in relation to age, trimester and gravidity 48

Table 4.7: CD4 count in relation to age, trimester and gravidity of HIV infected pregnant women 49

Table 4.8: Association between co-infection and intensity of infection. 51

Table 4.9: Univariate logistic regression predicting the occurrence of malaria parasitemia 53

Table 4.10: Univariate logistic regression predicting the occurrence of helminth infection 54

Table 4.11: Mean PCV values and anaemia classification in relation to infection status 56

Table 4.12: Association between anaemia and infections and the parasite densities 57

Table 4.13: Univariate logistic regression predicting the occurrence of anaemia 59

Table 4.14: Prevalence of infection in the subset of samples assayed for iron studies. 61

Table 4.15: Median (IQ) values of serum ferritin concentrations based on infection status 64

Content		Page

Table 4.16:	Median (IQ) values of serum ferritin concentrations of those in second and third trimesters based on infection status	65
Table 4.17:	Association between iron deficiency, anaemia and infections	67
Table 4.18:	Prevalence of anaemia and iron deficiency in relation to infection status	68
Table 4.19:	Median (IQ) values of serum iron concentrations based on infection status	71
Table 4.20:	Median (IQ) values of serum iron concentrations of those in second and third trimesters based on infection status	72
Table 4.21:	Association between abnormal iron levels, iron deficiency, anaemia and infections	74
Table 4.22:	Median (IQ) values of serum transferrin concentrations based on infection status	78
Table 4.23:	Median (IQ) values of serum transferrin concentrations of those in second and third trimesters based on infection status	79
Table 4.24:	Prevalence of infection by gestational age	81
Table 4.25:	Median (IQ) values of PIC concentrations of those with *Plasmodium* and its co-infection with helminths or HIV	84
Table 4.26:	Median (IQ) values of PIC concentrations of those in second and third trimesters with *Plasmodium* and its co-infection with helminths or HIV	85
Table 4.27:	Median (IQ) values of PIC concentrations of those with helminth and its co-infection with *Plasmodium* or HIV	88
Table 4.28:	Median (IQ) values of PIC concentrations of those in second and third trimesters with helminth and its co-infection with *Plasmodium* or HIV	89

Content		Page

Table 4.29:	Median (IQ) values of PIC concentrations of those with HIV and its co-infection with *Plasmodium* or helminths	92
Table 4.30:	Median (IQ) values of PIC concentrations of those in second and third trimesters with HIV and its co-infection with *Plasmodium* or helminths	93
Table 4.31:	Median (IQ) values of AIC concentrations of those with *Plasmodium* and its co-infection with helminths or HIV	95
Table 4.32:	Median (IQ) values of AIC concentrations of those in second and third trimesters with *Plasmodium* and its co-infection with helminths or HIV	96
Table 4.33:	Median (IQ) values of AIC concentrations of those with helminth and its co-infection with *Plasmodium* or HIV	98
Table 4.34:	Median (IQ) values of AIC concentrations of those in second and third trimesters with helminth and its co-infection with *Plasmodium* or HIV	99
Table 4.35:	Median (IQ) values of AIC concentrations of those with HIV and its co-infection with *Plasmodium* or helminths	101
Table 4.36:	Median (IQ) values of AIC concentrations of those in second and third trimesters with HIV and its co-infection with *Plasmodium* or helminths	102
Table 4.37:	Correlation coefficient values between *Plasmodium* infection alone, anaemia, iron status and cytokines	105
Table 4.38:	Correlation coefficient values in second trimester between *Plasmodium* infection alone, anaemia, iron status and cytokines	106
Table 4.39:	Correlation coefficient values between helminth infection only, anaemia, iron status and cytokines	107

Content		Page
Table 4.40:	Correlation coefficient values between HIV infection only, anaemia, iron status and cytokines	108
Table 4.41:	Correlation coefficient values in third trimester between HIV infection only, anaemia, iron status and cytokines	109
Table 4.42:	Correlation coefficient values between *Plasmodium* and HIV co-infection, anaemia, iron status and cytokines	110
Table 4.43:	Correlation coefficient values in third trimester between *Plasmodium* and HIV co-infection, anaemia, iron status and cytokines	111
Table 4.44:	Correlation coefficient values between no infection status, anaemia, iron status and cytokines	112
Table 4.45:	Correlation coefficient values in second trimester between no infection status, anaemia, iron status and cytokines	113

LIST OF FIGURES

Figure 2.1:	Life cycle of *Plasmodium falciparum*	8
Figure 2.2:	Life cycle of *Ascaris lumbricoides*	11
Figure 2.3:	Life cycle of *Trichuris trichiura*	12
Figure 2.4:	Life cycle of hookworm	13

ABBREVIATIONS

AIC –	Anti-inflammatory cytokines
EPG –	Eggs per gram
HIV –	Human Immunodeficiency Virus
IFN –	Interferon
IUGR –	Intrauterine Growth Retardation
IL -	Interleukin
LBW –	Low Birth Weight
MTCT –	Mother-to-child Transmission
PIBF –	Progesterone Induced Blocking Factor
PIC –	Pro-inflammatory cytokines
PTD –	Pre-term Delivery
SI –	Serum Iron
SF –	Serum Ferritin
STf –	Serum Transferrin
STH –	Soil-transmitted helminths
STI –	Sexually Transmitted Infection
TNF –	Tumor Necrosis Factor
WBC –	White Blood Cells
WHO -	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Plasmodium sp, helminths and human immunodeficiency virus (HIV) are major pathogens of global influence, particularly in developing countries causing malaria, helminthiasis and HIV/AIDS respectively. These diseases are also classified as poverty related diseases (PRDs) with children and expectant mothers as high risk groups. They are particularly endemic in Africa especially in sub-Saharan region where staggering statistics of morbidity and mortality have been reported.

In 2017, an estimate of 219 million malaria cases occurred worldwide with 80% of the world estimate occurring in sub-Saharan Africa and India (WHO, 2018). Twenty-five percent (25%) of these global cases occurred in Nigeria alone (WHO, 2018) where prevalence of 3.1% to 72% malaria cases have been reported among pregnant women from different locations in the country (Akanbi *et al.* 2006; Falade *et al*. 2008; Agomo *et al.* 2009; Raimi and Kanu, 2010; Bawa *et al.* 2014; Fana *et al.* 2015).

For soil transmitted helminths, the overall global burden was evaluated at 1.7 billion (Parija *et al.* 2017). Estimates of number of soil-transmitted helminths cases reported the overall global burdens for hookworm at 438.9 million, *Ascaris lumbricoides* at 819 million while 464.6 million people were infected with *Trichuris trichiura* (Pullan *et al.* 2014). Pregnant women in Nigeria are also vulnerable to STH infections with reports of 11.8% to 43.4% cases (Ozumba *et al*. 2005; Alli *et al*. 2011; Obiezue *et al*. 2013).

According to 2017 statistics on HIV, 36.9 million people are affected globally with 6.1 million of these reported cases in Western and Central Africa (UNAIDS, 2018). In Nigeria, 3.0% to 9.4% HIV cases in pregnancy have been reported (Ahmed *et al*. 2014; Okerentugba *et al*. 2015).

The alarming statistics of the burden of these diseases particularly the prevalence reports in pregnancy informed the Millennium Development Goal 5 (MDG 5) which

was set to improve maternal health with the aim of reducing maternal death ratio by seventy-five percent (United Nations, 2015). A lot of epidemiological data on the prevalence and pattern of these infections exist. However, there are still gaps in the existing knowledge for an elimination or eradication plan of these pathogens from the human race.

1.2 STATEMENT OF PROBLEM

Plasmodium, helminths and human immunodeficiency virus (HIV) are major agents of infectious diseases in the tropics with a heavy toll in developing nations especially in sub-Saharan region of Africa. These pathogens share similar geographical boundaries of distribution thus increasing the chances of co-infection of the causative organisms within a single host. The co-existence of these infections has become an interesting area of research particularly the immune-modulatory role of helminths in co-infection with the other pathogens. There are varying reports on the impact of co-infection in studies carried out among children, pregnant women, adults and even mice models.

In malaria and helminths co-infection, conflicting data have been reported on the associated risks of co-infection of these pathogens. Some studies in Uganda, Senegal and Nigeria reported increased risk of clinical malaria or an increased malaria parasite density (Spiegel *et al.* 2003; Le Hesran *et al.* 2004; Sokhna *et al.* 2004; Su *et al.* 2005; Adedoja *et al.* 2015) while others reported protection against cerebral malaria or a reduced malaria parasite density in studies carried out in Thailand and Ethiopia (Nacher *et al.* 2000; Degarege *et al.* 2012). Higher risk of low birth weights have also been reported in Nigeria, Ghana and Ethiopia among pregnant women with malaria and intestinal helminths co-infection (Egwunyenga *et al.* 2001; Graham *et al.* 2005; Degarege *et al.* 2010; Yatich *et al.* 2010).

Malaria and HIV co-infection studies have also been evaluated in different populations in Kenya, South Africa, Uganda, Malawi, Mozambique and Nigeria with reports of increased episodes of clinical malaria or an increased parasite density (van Eijk *et al.* 2003; Cohen *et al.* 2005; Kamya *et al.* 2006; Thigpen *et al.* 2011; Adeoti *et al.* 2012; Berg *et al.* 2014), increased viral load (Kublin *et al.* 2005) and higher occurrences of placental malaria (Perrault *et al.* 2009).

Some other studies in HIV and helminth co-infection in Kenya, Uganda and Zambia have reported increased chances of mother-to-child transmission of HIV (Gallagher *et al.* 2005), increased viral load (Brown *et al.* 2004) and increased HIV-1 replication (Modjarrad *et al.* 2005).

Among populations at risk of these infections are pregnant women (Hillier *et al.* 2008) posing high level risk for both the mother and the foetus. The success of pregnancy includes the physiologic wellness of the woman in being able to carry the pregnancy to term and deliver safely. However, these pathogens alter the natural course of pregnancy by modulations of the immune system and a predisposition to anaemia. The immune system components involved include human leukocyte antigen, immune cells, cytokines and chemokines (Chen *et al.* 2012). Cytokines, a diverse group of non-antibody proteins that act as mediators between cells are very influential in pregnancy as they have regulatory roles in initiation and maintenance of pregnancy (Kfutwah *et al.* 2009).

Furthermore, infections may positively or negatively impact the immune responses and further aggravate anaemia in pregnancy. Pregnancy requires T helper cells type 2 (Th 2) cytokine response for a positive pregnancy outcome (Desai 2007; Sykes *et al*. 2012). However, inflammation and infections may result in dominance of Th 1 cytokines and these are associated with 'small for gestational age' babies, intrauterine growth retardation, recurrent spontaneous abortion and preterm delivery (Raghupathy *et al*. 2000). More so, about 8% to 16% maternal deaths have been attributed to anaemia. A number of works in journal articles have evaluated the prevalence of anaemia in the presence of infection (Muhangi *et al*. 2007; Ouma *et al*. 2007; Baidoo *et al*. 2010; Oladeinde *et al*. 2012). However, a detailed assessment of anaemia involves evaluation of the packed cell volume (haematocrit) and measures of iron status parameters such as ferritin, transferrin, total iron binding capacity etc.

1.3 JUSTIFICATION FOR THE RESEARCH

Malaria, helminthiasis and HIV are of public health importance in Nigeria where high transmission rates of the pathogens have been reported at different times. Hence, continuous evaluation of their prevalence as a tool for measuring efficacy of government intervention programmes is required. In this study, the target population were pregnant women being a high-risk group for these infections with implications for both maternal and neonatal health. The effects of these infections on maternal health include alterations in the cytokine profile and changes in the iron status which are major determinants in ensuring a pregnancy is carried to term.

Cytokines are polypeptides released from cells which on binding to specific cellular receptors, alter cell growth, differentiation and/or function (Clark, 1989). They have been associated with initiation and maintenance of pregnancy (Desai 2007; Sykes *et al.* 2012). Distortions in the systemic cytokine profile of pregnant women increases the likelihood of adverse birth outcomes and this has been reported in women with reproductive failure (Mahdi, 2011). A number of studies have evaluated these changes in cytokine profile in mono infection of *Plasmodium,* intestinal helminths and HIV. Studies from India, Nigeria and Saudi Arabia have evaluated levels of IFN-γ, IL-2, IL-5, IL-6 and IL-12 in pregnant women with *Plasmodium* only (Prakash *et al.* 2006; Nmorsi *et al.* 2010a; Nasr *et al.* 2014). A study from Nigeria also measured levels of TNF-α, IL-6, IL-8 and IL-10 in pregnant women infected with helminths only (Arinola *et al.* 2015; Olateru-Olagbegi *et al.* 2018) while some other studies in USA and Brazil evaluated levels of TNF-α, IFN-γ, IL-1α, IL-2, IL-4, IL-6, IL-8 and IL-10 in HIV infected pregnant women (Sutton *et al.* 2004; Sachdeva *et al.* 2008; Richardson and Weinberg, 2011; Hygino *et al.* 2012; Tudela *et al.* 2014).

However, there are fewer reports on the immunological interplay of their co-infections. A study in Brazil reported levels of thirteen cytokines and three chemokines in pregnant women with malaria and helminths co-infection using a magnetic bead-based multiplex assay (Sánchez-arcila *et al.* 2014). In Nigeria, a study also determined levels of IFN-γ and IL-10 in pregnant women with malaria and HIV co-infections (Adeoti *et al.* 2015). However, there is deficit of data on helminths and HIV co-infection in the pregnant population. A study conducted in South Africa reported levels of IgE and lymphocyte activation markers in HIV-infected adults (Mkhize-Kwitshana *et al.* 2011). These investigations have linked the forms of cytokine secretions with disease

outcome but the results are reportedly influenced by variations in study population (Sinha *et al.* 2010). Hence, it is difficult to generalize these relationships for all cohort studies.

Iron is highly essential with an increase in demand in pregnancy. However, there is a predisposition to anaemia in women harbouring parasitic infections (WHO, 2011a). Indices used in the evaluation of maternal iron status include haemoglobin concentration, haematocrit, ferritin, soluble transferrin receptor, bone marrow aspiration, transferrin saturation, serum iron, total iron binding capacity (TIBC), zinc protoporphyrin etc.

A number of studies have evaluated levels of haematocrit, packed cell volume or haemoglobin in pregnancy among those with malaria (Onyenekwe *et al.* 2005; Agan *et al.* 2010; McClure *et al.* 2014), intestinal helminths (Getachew *et al.* 2012; Obiezue *et al.* 2013) or HIV (Manyanga *et al.* 2014; Melku *et al.* 2014). Malaria is a major cause of anaemia in pregnancy as the pathogen is an intraerythrocytic parasite destroying infected red cells during schizont rupture and also unparasitized red cells (White, 2018). Likewise, anaemia in HIV patients is linked to increased red blood cell (RBC) destruction, reduced RBC production and ineffective RBC production (Volberding *et al.* 2004). However, fewer studies reported ferritin levels in pregnancy among those with malaria (Ndyomugyenyi *et al.* 2008; Eteng *et al.* 2010), intestinal helminths (Nurdiati *et al.* 2001) and HIV (Friis *et al.* 2001; Kupka *et al.* 2007). Some research works in pregnancy have also monitored the disparities in the concentrations of some of these iron indices amongst those with or without infections (Paiva *et al*. 2007; Buseri *et al*. 2008). Specifically, assessment of ferritin, transferrin and iron levels in relation to infection have oftentimes been evaluated in children and non-gravid subjects (Stoltzfus *et al.* 1997; Odunukwe *et al.* 2000; Aini *et al.* 2007; Adebara *et al.* 2011; Banjoko *et al.* 2012) with fewer reports among pregnant women. Hence, a need to review some of these markers in pregnancy for an in-depth assessment of their contributions to anaemia.

The iron status and cytokine profiles of peripheral blood of pregnant women infected with *Plasmodium sp*, intestinal helminths and HIV need to be fully evaluated. This is particularly important in tropical countries where the diseases are widespread and may coexist in pregnancy. In Nigeria, these host factors have not been fully explored and there is also a dearth of data on trimester-based interaction of these diseases and host

factors. An assessment of multiple parameters to determine iron status in pregnancy together with the evaluation of an array of cytokines will provide a better insight on the impact of infections on pregnancy course. The findings of this research will also add to knowledge that could be beneficial when designing trials for vaccine efficacy in regions with high occurrences of co-infections.

1.4 AIM OF THIS STUDY

This study was set to evaluate the role of single and co-infections of *Plasmodium*, intestinal helminths and HIV on maternal iron status and cytokine expression in peripheral blood of pregnant women.

Specific objectives

The specific objectives are to:

1. Determine the prevalence of malaria parasitemia and helminth infections among HIV-infected and non-infected expectant mothers.
2. Evaluate the likely risk factors associated with these infections among the study population.
3. Evaluate iron status of the pregnant women in relation to infection using different iron status indices.
4. Quantify the cytokines produced in peripheral blood at different trimesters in response to mono and co-infections of malaria, HIV and helminth.
5. Correlate iron status with cytokine profile of parturient women.

CHAPTER TWO

LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF MALARIA

The malaria parasite is transmitted to humans through a vector, the female *Anopheles* mosquito. There are five major types of the parasite species that affect humans namely *Plasmodium falciparum, P. malariae, P. ovale, P. vivax* and *P. knowlesi*. In endemic regions of tropical Africa, *P. falciparum* is the prevalent species. It is known to cause severe infections that may lead to death if not promptly treated. The malaria parasite resides in red blood cells making transmission through blood transfusion, shared use of blood contaminated needles or syringes or organ transplant possible. It may also be transmitted congenitally before or during childbirth (CDC, 2019a). A schematic representation of the life cycle of *P. falciparum* is shown in Figure 2.1.

In literature, a couple of risk factors have been reported for malaria. In Gambia, it was found that mud wall and poor cleanliness were potential risk factors (Koram *et al.* 1995). Assessment of occupation and malaria infection in Zaire showed higher cases of malaria among the low paid, industrial or unskilled workers (Tshikuka *et al.* 1996). Furthermore, in a survey implemented in Nigeria, low birth weights and higher parasitemia were observed among those attending low socio-economic status hospitals (Olowu *et al.* 2000). Low socio-economic status hospital was defined as maternity hospital while university hospital was defined as middle or high socio-economic status hospital. In a survey within the Niger-Delta region of Nigeria, the presence of stagnant water in the vicinity of the households was a key predisposing factor for malaria (Madukaku *et al.* 2012).

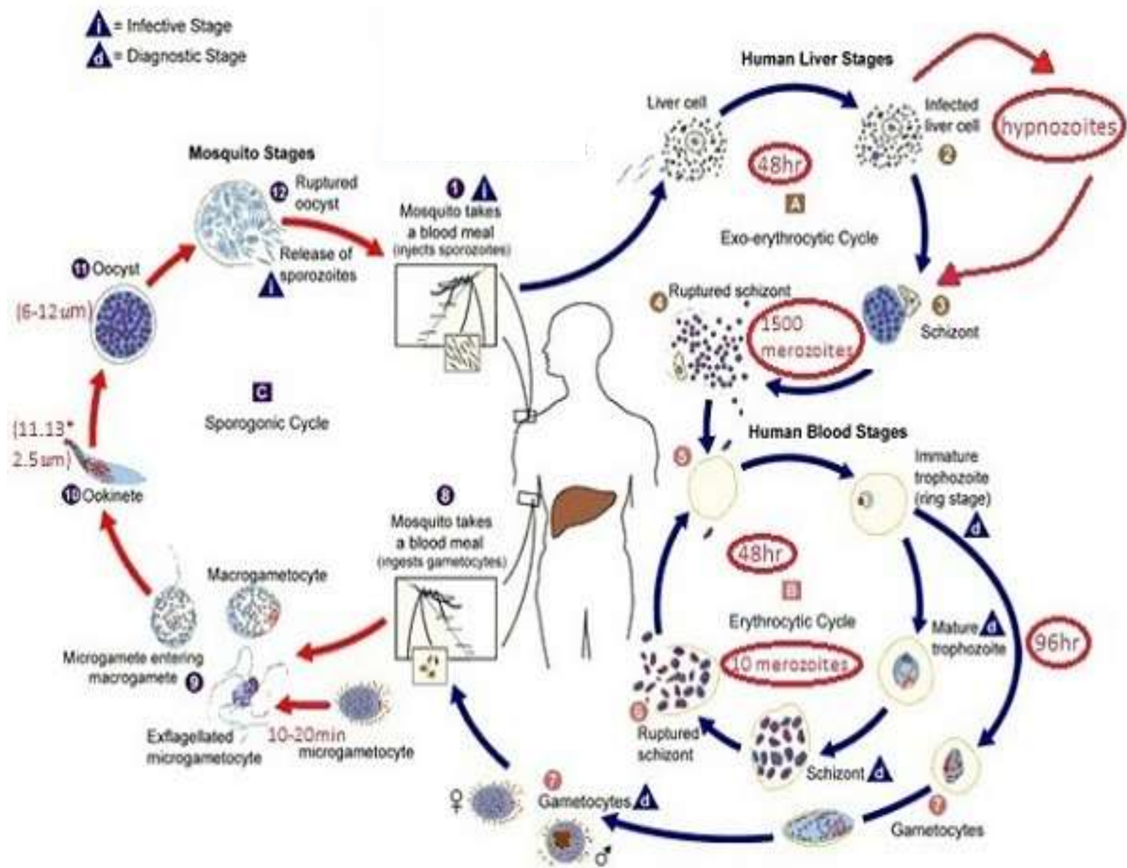

Figure 2.1: Life cycle of *Plasmodium falciparum*

Source: CDC - *http://www.cdc.gov/dpdx/malaria/*

The burden of malaria remains alarming in Africa with the sub-Saharan region being the most affected. About 219 million malaria cases and 435,000 related deaths occurred globally in 2017 with a quarter of these global malaria cases and 19% mortality due to malaria occurred in Nigeria alone (WHO, 2018). Pregnant women and children <5years bear a heavy toll of infections particularly the malaria disease. Malaria, being the most prevalent parasitic infection among pregnant women, it has an estimated minimum of one in four expectant mothers harbouring the parasites at childbirth in areas of stable transmission in Africa (Desai *et al.* 2007). The burden of malaria in Africa has been a major challenge in the last few decades with the effects felt by the mother, foetus and neonates (Hamer *et al.* 2009). In areas where malaria is widely distributed, primigravidae have a higher susceptibility to the infection and have been reported to harbour a higher prevalence and density of parasitemia in peripheral (Hamer *et al.* 2009) and placental blood (Brabin *et al.* 2004). In Nigeria, 3.1% to 72% cases of malaria have been reported among pregnant women from different locations in the country (Akanbi *et al.* 2006; Falade *et al*. 2008; Agomo *et al.* 2009; Raimi and Kanu, 2010; Bawa *et al.* 2014; Fana *et al.* 2015).

The 2001-2010 United Nations Decade to Roll Back Malaria Report attributed 10,000 maternal deaths, 8-14% of all low birth weight babies (LBW) and 3-8% of all infant mortality in certain parts of Africa to malaria in parturition. *P. falciparum* is reported to be a key cause of LBW in first pregnancies (Brabin *et al.* 2004). LBW often occurs in babies with malaria parasitemia in a survey done in Nigeria (Ayoola *et al.* 2012). The increase in number of LBW was found to be higher among primigravidae and it ranged from <10% in low transmission areas to >50% in high endemic areas (Brabin *et al.* 2004). However, age associated immunity is also believed to have a crucial part in the susceptibility of pregnant women in regions of high and stable transmission (Desai *et al.* 2007). Although symptomatic presentations of malaria are uncommon, the consequences are usually severe resulting in maternal anaemia, LBW infants, intrauterine growth retardation (IUGR) and infant mortality. In regions of unstable malaria transmission, the resultant effects of MIP are more serious with higher risk of symptomatic malaria, severe and complicated malaria, severe anaemia, preterm deliveries, abortion and stillbirths (Desai *et al.* 2007). Among expectant mothers at the point of enrolment in ante-natal clinic in coastal Kenya, maternal anaemia was found to be related to *P. falciparum* infection (McClure *et al.* 2014). In a study in Uganda,

malaria in pregnancy was also linked to higher incidence of childhood malaria (Ndibazza *et al.* 2013). Malaria parasitemia in maternal peripheral blood likewise increased the chance of pre-term birth in a work done in Nigeria (Falade *et al.* 2010).

2.2 EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHS INFECTIONS (STHs)

Soil-transmitted helminths are also known as intestinal helminths. The four main species that affect humans are *Ascaris lumbricoides, Trichuris trichiura* and the hookworms: *Ancylostoma duodenale* and *Necator americanus*. The life cycles of these species are shown in Figure 2.2, 2.3 and 2.4. The primary means of transmission to humans is ingestion of eggs passed through faeces in soil, water or food. Hookworms infect directly through the soles of the feet particularly around open defaecation sites and latrines when people walk barefoot. Helminth infection is widely distributed in Africa and pregnant women are among the vulnerable groups (Hillier *et al.* 2008). Prediction of the distribution of these species using physical factors like rainfall, temperature and altitude suggests that the prevalence of *A. lumbricoides* and *T. trichiura* is highest in equatorial, eastern Madagascar, southeast Africa as well as central and west Africa. However, hookworm is more widespread across the nations of the continent (Brooker *et al.* 2007).

Environmental conditions, socioeconomic status cum living conditions affect the distribution of these parasitic infections (Brooker *et al.* 2007; Getachew *et al.* 2012; Madukaku *et al.* 2012; Gyawali *et al.* 2013). Young age and no formal education were identified as risk factors for all helminth species in a research work in Uganda (Woodburn *et al.* 2009) where crude associations were found between some helminth species and gravidity, walking barefoot, lack of toilet etc. Similarly, in Nepal, a close association existed between poor sanitary practices, lack of portable water source, level of education, living conditions and susceptibility to helminth infections (Gyawali *et al.* 2013). In Northern Ethiopia, gender, age, handwashing habit, use of latrines, swimming and cleanliness of fingernails were all associated with helminth infection among school children (Abera and Nibret, 2014).

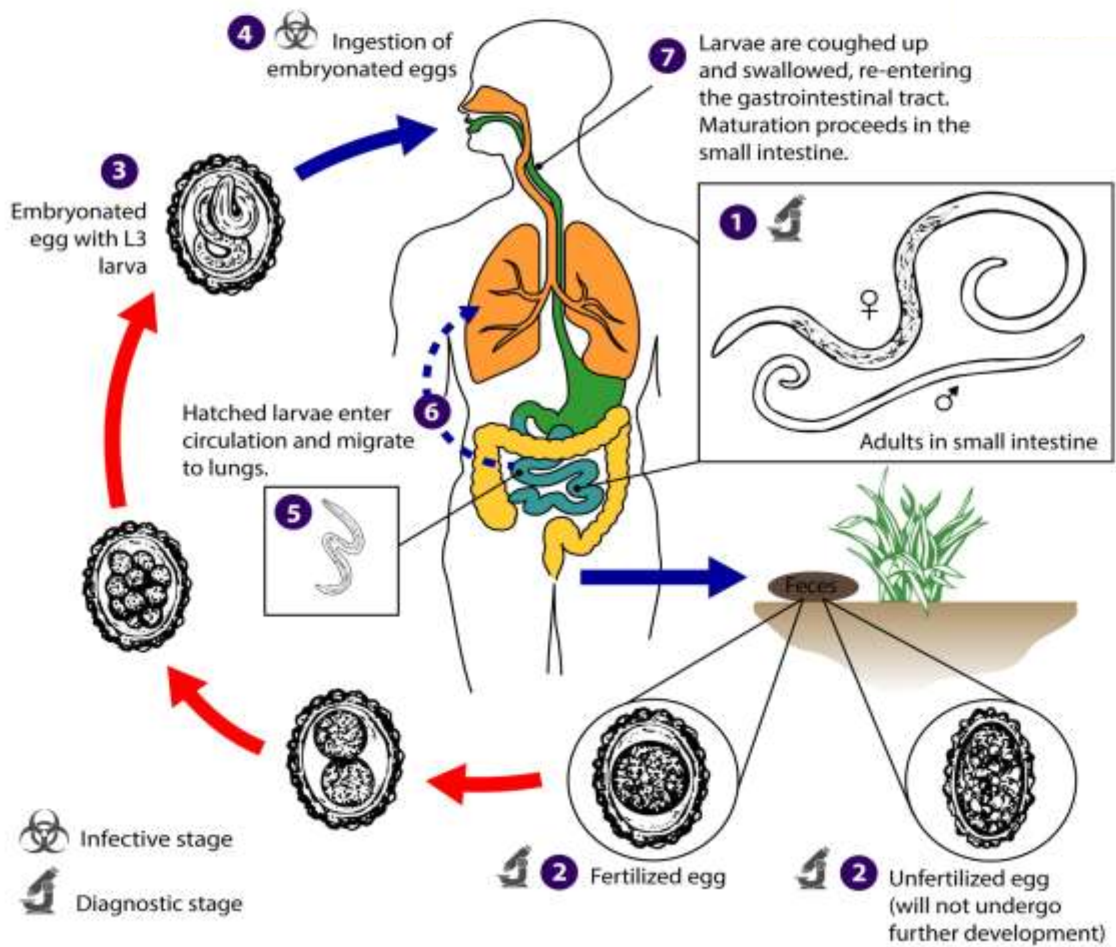

Figure 2.2: Life cycle of *Ascaris lumbricoides*

Source: CDC - *http://www.cdc.gov/parasites/ascariasis/biology.html*

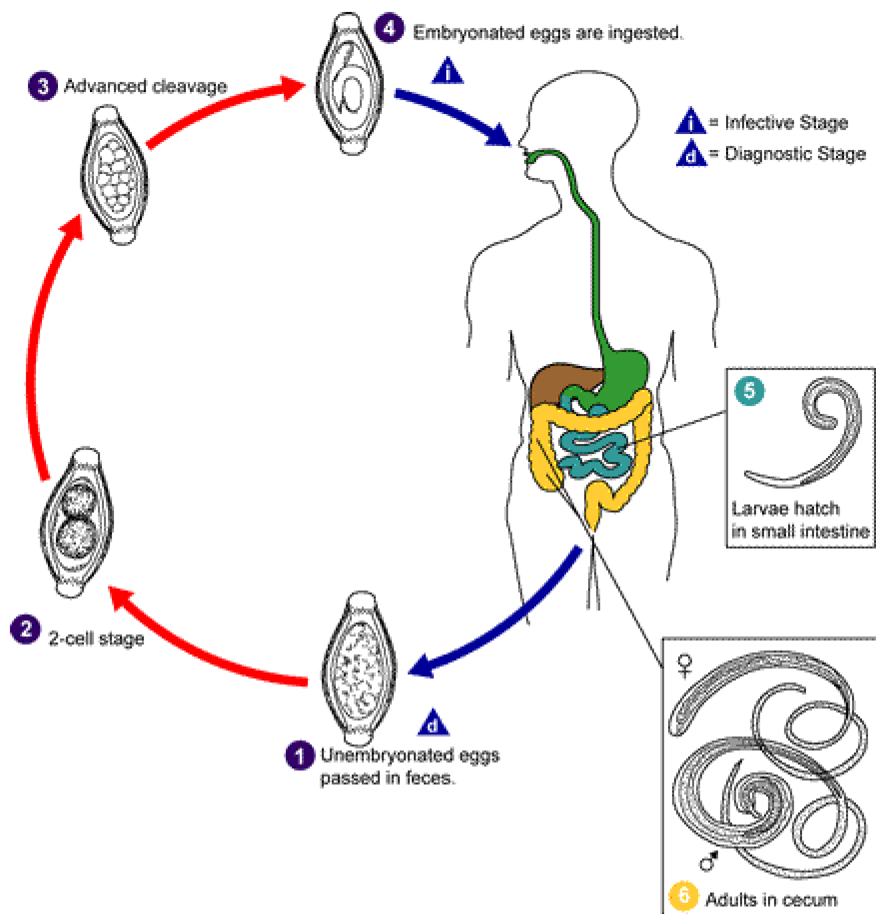

Figure 2.3: Life cycle of *Trichuris trichiura*

Source: CDC - *http://www.cdc.gov/parasites/ascariasis/biology.html*

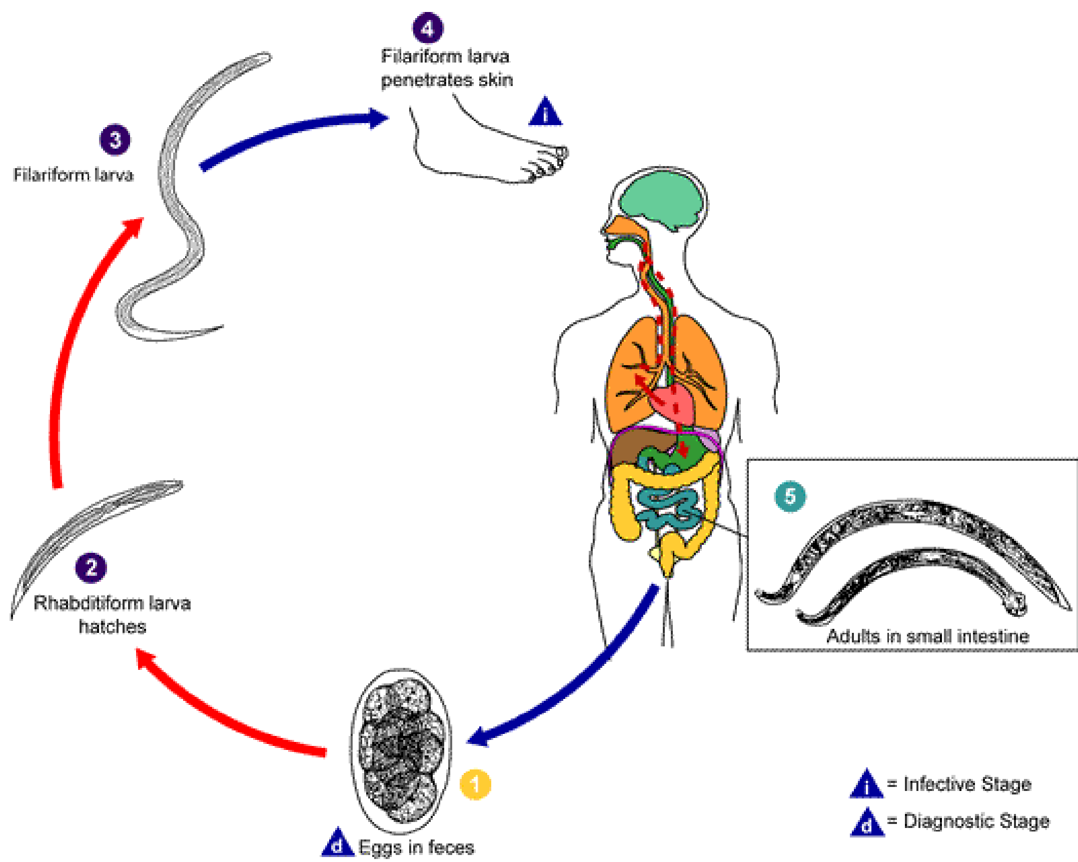

Figure 2.4: Life cycle of hookworm

Source: *http://www.cdc.gov/parasites/hookworm/biology.html*

The risk of helminthiasis in pregnancy was higher in rural settings due to low socioeconomic status, poor environmental sanitation and inappropriate waste disposal methods (Wekesa *et al.* 2014). In Nigeria, not washing hands after defaecation and drinking water from the stream were associated with helminthiasis (Odinaka *et al.* 2015). Recently, contamination of soils with helminth eggs due to open defaecation practices was a reported risk factor for helminthiasis (Oyebamiji *et al.* 2018).

The statistics of the number of people harbouring intestinal helminths is staggering. About 1.5 billion people have soil-transmitted helminths (STHs) and a higher percentage of these population are in sub-Saharan Africa, China, the Americas and East Asia. In 2010, inferences were made from a pool of available data and an estimate of over one billion people were reportedly infected with helminths globally. The specific prevalence of common helminth infections reported were 438.9 million cases of hookworm infection, 819 million (14.5%) people infected with *A. lumbricoides* and 464.6 million (8.3%) people infected with *T. trichiura*. From these global figures, 117.7 million (13.6%) people are infected with hookworms, 117.9 million (13.6%) people infected with *A. lumbricoides* and 100.8 million (11.6%) people infected with *T. trichiura* in sub-Saharan Africa (Pullan *et al*. 2014). In Nigeria, more studies have focused on helminthiasis in children relative to pregnant women but a few studies have reported 11.8% to 43.4% cases of helminthiasis in pregnancy (Ozumba *et al*. 2005; Alli *et al.* 2011; Obiezue *et al*. 2013).

Helminth infections particularly hookworms have been identified as one of the causal agents of anaemia in parturition. A study done in Nigeria reported a relationship between anaemia and helminthic infection among pregnant women (Obiezue *et al.* 2013). Furthermore, reduced immune response to postnatal immunization was also found in mothers with helminth infection (Sabin *et al.* 1996). Helminth infection further posed a higher risk of infection with *P. falciparum* as shown in a work carried out in Ghana (Yatich *et al.* 2009). In a research executed in Uganda, hookworm infection in pregnancy increased odds of asymptomatic parasitemia and clinical malaria in the children relative to children born by uninfected mothers (Ndibazza *et al.* 2013).

2.3 EPIDEMIOLOGY OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

There are three basic ways of HIV transmission: unprotected sex, donated blood or blood products and from infected mother to her child. Transmission occurs when body fluids of an infected person come in contact with a mucuos membrane or broken tissue or when the fluid is directly injected into the blood stream from a needle or syringe. These body fluids are vaginal fluids, blood, pre-seminal fluid, semen, rectal fluids, and breast milk. The rectum, vagina, penis and mouth are lined with mucous membranes (CDC, 2019b).

A study extensively evaluated various factors associated with HIV infection. Some identified risk factors were alcohol consumption, previous history of sexually transmitted infection (STI), increased gravidity, widowed or divorced marital status and history of blood transfusion (Woodburn *et al.* 2009). Additional risk factors from studies in Nigeria were history of having sex for commercial purpose, no previous HIV screening, lack of awareness of other means of HIV transmission except sexual intercourse, homosexuality, multiple sex partners, use of intravenous drugs and sharing of sharp objects (Gomwalk *et al.* 2012; Okerentugba *et al.* 2015).

Globally, about 36.9 million people have HIV of which 6.1 million reside in Western and Central Africa with AIDs-related deaths estimated at 280,000 in the region (UNAIDS, 2018). Moreover, three-fourths of newly discovered infections are among girls aged 15-19 years and the likelihood of women within the ages of 15-24 years being positive for HIV is double (UNAIDS, 2018). Studies among pregnant women in different locations in Nigeria showed prevalence of 3.0% to 9.4% (Ahmed *et al.* 2014; Okerentugba *et al.* 2015).

A high prevalence of 45% mother-to-child transmission (MTCT) rate of HIV occurred in a study among HIV-infected women in a work done in Nigeria (Odaibo *et al.* 2006). Similarly, HIV predisposes to maternal mortality and a survey conducted in Nigeria reported that 24.5% cases of maternal death was HIV-related (Onakewhor *et al.* 2011). Similarly, A longitudinal research in Uganda reported increased chances of being underweight in babies born to women living with HIV (Muhangi *et al.* 2013).

2.4 CO-INFECTION

The similarity in geographical distribution of *Plasmodium,* intestinal helminths and HIV particularly in Africa increases the chances of them co-existing within a single host. In recent years, more studies are addressing the implication of co-infection of these infectious agents on epidemiological pattern, immune response, drug and vaccine efficacies. Much interest has focused on a potential link between helminth infections and other infectious diseases with discussions more on the protective or aggravating effects of helminth co-infection with other infectious diseases.

2.4.1 Malaria and Helminth Co-infection

Malaria and helminth infections occur in various parts of the tropical region. Hence, co-infection of the two species are frequently observed. A number of publications have been generated from this field of research particularly on the epidemiology of these infections in different cohorts of people (Egwunyenga *et al.* 2001; Degarege *et al.* 2010). In a research done in Nigeria, more than 45% of *Plasmodium*-infected parturient women in the study also had multiple helminth species (Egwunyenga *et al.* 2001). Some studies in humans have raised the probability of helminth infection increasing the susceptibility to malaria. In a study among children, there was reduction in risk of clinical malaria among those with no intestinal nematodes infection compared with those infected (Spiegel *et al.* 2003). Similar finding of higher occurrence of malaria was reported among children with *Schistosoma mansoni*, particularly in those with the highest helminth egg counts (Sokhna *et al.* 2004). A survey in Senegal also reported that *Ascaris* was associated with increased number of severe malaria cases (Le Hesran *et al.* 2004). Conversely, some works have reported that helminth infections play a protective role against malaria. In Thailand, *Ascaris* infection reportedly protects against cerebral malaria and renal failure (Nacher *et al.* 2000; Nacher *et al.* 2001). Similarly, a study carried out in Malawi reported that those infected with *S. haematobium* had reduced malaria parasite densities compared with those with *Plasmodium* only (Thigpen *et al.* 2011).

Different experimental works have added to our understanding of the impact of one on the other. Co-infection of the filarial worm, *Litosomoides sigmodontis* with malaria resulted in loss of weight but no increased count in malaria parasitemia (Graham *et al.*

2005) while co-infection of *P. chabaudi* and a gastro-intestinal nematode, *H. polygyrus* increased malaria parasite count and number of deaths due to malaria (Su *et al.* 2005). Hence, both human and murine models of malaria and helminth co-infections are strongly linked to helminth species, strains of *Plasmodium*, genetic makeup of the mice, age of the population being studied, stage and severity of infection (Helmby, 2009).

Despite the depth of information on the outcomes of combined infections of malaria and intestinal helminths, few works evaluated this among gravid women. In a research carried out in Ghana, Yatich *et al*. found 3-fold risk of LBW among women with co-infection of malaria and helminths relative to the uninfected women (Yatich *et al.* 2010). In an investigation implemented in an hospital in Jos, Nigeria, co-infection of *Plasmodium* and intestinal helminths resulted in neonates with lower mean birth weights relative to those with *Plasmodium* only (Egwunyenga *et al.* 2001). A study among hospital outpatients in Ethiopia also showed increased number of cases of anaemia and low weight status among those with co-infection of *Plasmodium* and helminths parasites (Degarege *et al.* 2010).

2.4.2 Malaria and HIV Co-infection

Malaria and HIV combined infection is a major challenge in sub-Saharan Africa, Southeast Asia and the Indian subcontinent where the majority of those affected live. The severity of these interactions is compounded by the similarity in the global distribution of these diseases (Hochman and Kim, 2009).

Studies have shown that individuals with HIV infection are more prone to recurrent bouts of acute malaria (Kamya *et al.* 2006), more occurrences of severe cases of malaria and mortality in children and adult (Grimwade *et al.* 2003; Cohen *et al.* 2005; Berg *et al.* 2014) and higher malaria parasite densities (Thigpen *et al.* 2011; Berg *et al.* 2014). In addition, HIV-infected people have increased viral load during episodes of parasitemia (Kublin *et al.* 2005), resulting in a likely increase in the possibilities of HIV transmission. Among HIV positive pregnant women, higher rates of symptomatic malaria (van Eijk *et al.* 2003), placental malaria, and malaria cases in children (Perrault *et al.* 2009) have been reported. However, the possibility of malaria affecting MTCT of HIV is unknown though the increase in viral load is established in malaria

infection (Kublin *et al.* 2005). It is not clear if this infers higher transmission rates of HIV. Moreover, it is also not yet certain if HIV enhances malaria transmission rate, though it is established that HIV increases the malaria parasite density. In Kenya, a study reported higher parasite densities in the placenta as well as higher rates of malaria transmission in malaria infected HIV positive women compared to the women not infected with HIV during antenatal period of pregnancy (Perrault *et al.* 2009).
While some interplay between malaria and HIV have been investigated, these associations are inconclusive from an epidemiological point of view.

2.4.3 Helminth and HIV Co-infection

Fewer works that have evaluated the number of cases of HIV and helminth co-infection have reported low prevalence rates. In a survey done in Calabar, Nigeria, the cases of intestinal helminths among HIV-infected individuals was 8.7% (Etok *et al.* 2010). Another research in Kenya reported 19.3% HIV-1 prevalence among adults (Walson *et al.* 2010). Some studies also tested the relationship between helminths and HIV infections in parturition and showed that the association in both infections is likely helminth species dependent. In Kenya, HIV infected women were more susceptible to lymphatic filariasis (Gallagher *et al.* 2005) while another study in Malawi showed they were less likely to have *Schistosoma haematobium* infection (Thigpen *et al.* 2011). Another study showed that living in Western Kenya increased the probability of *A. lumbricoides* and *S. mansoni* infections compared with those living in urban Nairobi (Walson *et al.* 2010). In addition, they found no remarkable changes in CD4 cell count of individuals infected with helminths and those without. However, restriction of data to those with *S. mansoni* or *Strongyloides stercoralis* infection showed associations between these infections and a higher mean log viral load (Brown *et al*. 2004). A research in Rwanda also showed expectant mothers with detectable viral load to be more at risk of *A. lumbricoides infection* than those without detectable viral load. However, those with CD4 count less than 350 cells/mm^3 were prone to being infected with different species of helminths compared with those with counts greater than 350 cells/mm^3 (Ivan *et al.* 2013). A study in Kenya also evaluated the impact of this co-infection on birth outcome and reported that MTCT of HIV was significantly higher among those with co-infection of helminths (Gallagher *et al.* 2005).

2.5 ANAEMIA, IRON STATUS AND PREGNANCY

Anaemia is a state wherein there are fewer red blood cells (RBCs) resulting in less oxygen supply that cannot adequately provide for the body's needs (WHO, 2011a). Globally, iron deficiency (ID) is the most frequent cause of anaemia but other sources of anaemia are inflammation, nutritional deficiencies, inherited or acquired blood disorders, inherited or acquired blood disorders and parasitic infections (WHO, 2011a).

According to World Health Organization (WHO) recommendation for determination of anaemia and assessment of severity in pregnancy, haemoglobin concentrations of 110g/L or higher was defined as normal. Mild anaemia was categorized as haemoglobin concentration of 100-109g/L, moderate anaemia as 70-99g/L while severe anaemia was defined as lower than 70g/L (WHO, 2011a). Measures of iron status include: haemoglobin concentration, haematocrit, ferritin, soluble transferrin receptor, bone marrow aspiration, serum iron, total iron binding capacity (TIBC), transferrin saturation, zinc protoporphyrin and red blood cell markers. However, according to the report of a WHO/CDC working group, five indicators were selected to measure iron status (WHO, 2007). These include:

a. **Haemoglobin concentration:** Haemoglobin concentration, as a measure of anaemia varies among different population cohorts and in individuals living in different altitudes. People living in regions of high altitudes reportedly have higher concentrations of haemoglobin (WHO, 2011a). In the tropics, reported cases of anaemia range was 16.6% to 76% among expectant mothers (Idowu *et al*. 2005; Gwarzo and Ugwa 2013; Melku *et al*. 2014; Olatunbosun *et al*. 2014). In another study that compared gravid and non-gravid women, the cases of anaemia was greater among the parturient cohort (Buseri *et al.* 2008). Another study reported that the iron nutritional status of expectant mothers with mild anaemia or ID showed negligible impact on the iron status of their offspring (Paiva *et al.* 2007).

b. **Mean cell volume:** MCV is the mean volume of the individual red blood cell (Khan *et al.* 2013). This shows whether red blood cells are microcytic or macrocytic which is an indication of iron deficiency or megaloblastic anaemia respectively. It has been shown not to change significantly during pregnancy (Mutua *et al.* 2018).

c. **Zinc protoporphyrin:** This indicates a scarcity in iron supply in the last stage of making haemoglobin. It is a test of the acuteness of iron deficiency measured by excess formation of zinc protoporphyrin. However, it has been reported to be an unreliable diagnostic tool for differentiating between pregnant women with or without iron deficiency (Mwangi *et al.* 2014).

d. **Soluble transferrin receptor:** This shows the intensity of erythropoiesis and the need for iron. It is a good marker only after serum ferritin levels have been depleted and no other identifiable cause of abnormal erythropoiesis. It is also a valid index of ID in parturition (Carriaga *et al.* 1991). It is a more definitive marker when there is functional ID (Skikne *et al*. 1990).

e. **Serum ferritin:** This measures iron in body stores in the absence of concurrent infections. This is the most sensitive iron status determinant (Skikne *et al*. 1990). Iron stores exists primarily as ferritin in the body. When there is no inflammation, plasma or serum levels of ferritin is directly linked with total body iron stores (WHO, 2011b). A study in Seychelles showed that iron deficiency in mothers at the time of delivery resulted in neonates with low serum ferritin values compared with neonates from non-deficient mothers (Duffy *et al.* 2009).

2.5.1 Infections associated anaemia and changes in iron status in pregnancy

Aside nutritional deficiency, infection is also a major source of anaemia (WHO, 2011a). In a survey of gravid mothers in Nigeria, 40.2% of the women were anaemic and this was reported to be due to infection or inflammatory processes (Buseri *et al.* 2008). Anaemia prevalence of 58.4% was found among those with asymptomatic malaria in Nigeria (Agan *et al.* 2010). Another research in Nigeria found no considerable change in haematocrit values among expectant mothers with asymptomatic malaria and those without malaria parasitemia. However there was inverse correlation between malaria parasite density and haematocrit values (Onyenekwe *et al.* 2005). Similar inverse relationship was found between serum transferrin concentration and haematocrit values among those infected with malaria parasites (Onyenekwe *et al.* 2005). Another study among Beninese women also associated malaria and helminth infestations with low maternal haemoglobin concentrations (Ouedraogo *et al.* 2013). Similarly, pregnant mothers residing in rural areas of Ethiopia and infected with *Plasmodium* or intestinal helminths were at higher risks of being anaemic (Getachew *et al.* 2012).

In a study carried out among children, no relationship was found between ferritin and malaria at parasite densities <1000 parasites/μL blood. They likewise found a slight increase in ferritin concentration as malaria parasitemia increased above this level (Stoltzfus *et al.* 1997). This positive correlation in ferritin concentration and malaria parasite density was also reported among apparently healthy adults with malaria parasitemia (Odunukwe *et al.* 2000). Another study among pregnant women also reported association between malaria parasitemia and serum ferritin concentration (Ndyomugyenyi *et al.* 2008). Significant increases in serum transferrin and serum iron among malaria positive pregnant women relative to the uninfected have likewise been reported (Eteng *et al.* 2010). However, another investigation in Cameroon showed substantial reduction in serum iron among malaria patients (Gouado *et al.* 2008).

A research among pregnant women in Kenya detailed that STH infection was not associated with severe form of anaemia (van Eijk *et al.* 2009). However, they found hookworm infection of ≥1000 eggs/gram faeces to be associated with an average reduction of 1.5g/dL in haemoglobin (van Eijk *et al.* 2009). In another study from coastal Kenya, moderate or severe forms of anaemia at booking in those registered at antenatal clinic was linked to high intensities of hookworm and *P. falciparum*

infections. This study also found that moderate or severe maternal anaemia at booking or at childbirth was associated with foetal anaemia (McClure *et al.* 2014). In children, higher chances of severe trichuriasis and severe ascariasis was reported among children with low haemoglobin values, low serum iron or low serum ferritin (Aini *et al.* 2007). Likewise, school children infected with intestinal helminths were found to have lower serum ferritin levels in a survey executed in Nigeria (Adebara *et al*. 2011).

Among women with HIV, anaemia cases of 54% was reported in Tanzania and poor adherence to the use of co-trimoxazole in pregnancy resulted in the increased susceptibility to anaemia (Manyanga *et al*. 2014). Manyanga and colleagues also found a higher number of anaemia cases among those with co-infection of malaria and HIV relative to those with HIV only. Those who had malaria were 10.4 times more likely to be anaemic compared with those without malaria (Manyanga *et al.* 2014). Another study among pregnant women in Ethiopia also associated high rate of anaemia with HIV seropositivity, hookworm infection or having a chronic disease among expectant mothers in Ethiopia (Melku *et al.* 2014). In another study, HIV infection resulted in slightly lower iron stores among women with asymptomatic presentation. Although they likewise reported an unusual deficit in haemoglobin, it was proposed that this could likely be caused by low iron intake and absorption offsetting the shift in iron from circulating erythrocytes to the stores (Friis *et al.* 2001).

Similarly, a negative association between haemoglobin level and plasma viral load has been reported (Semba *et al.* 2001). However, in a further assessment of the relationship between iron status and markers of HIV severity, it was found that the iron status of the parturient women was not related to indicators of HIV severity (Semba *et al.* 2001). On the contrary, a work done in Tanzania among women with HIV showed there was a correlation between serum ferritin and viral load (Kupka *et al.* 2007). Among HIV infected individuals, serum iron was remarkably elevated among those infected with HIV relative to those that are negative. There was also an inverse relationship between serum iron concentration and CD4 lymphocytes (Banjoko *et al.* 2012).

2.6 ROLE OF IMMUNE SYSTEM IN SUSTENANCE OF PREGNANCY TO FULL TERM

Cytokines constitute a portion of the immune system involved in the initiation and sustenance of pregnancy with a bias for T-helper cells 2 (Th 2) responses (Desai *et al.* 2007). T-helper cells are grouped according to their cytokine production. Th 1 cells produce interferon – gamma (IFN-γ), turn on macrophages and are actively involved in the fight against infectious agents such as malaria parasites and HIV that invade the cells. Conversely, Th 2 cells produce IL-4, IL-5 and IL-13 and are responsive in mast cells activation, eosinophils and secretion of more IgE antibodies (Helmby, 2009). Due to the production of these cytokines by a varied number of cells, these responses are preferably referred to as Th 1-type or pro-inflammatory cytokines and Th 2-type or anti-inflammatory cytokines. Although there are other types of Th cells, the prime subsets of totally differentiated CD4 T cells are Th 1 and Th 2 cells and their peculiar roles in immune responses is associated with their distinctions in their cytokine production patterns (Table 2.1). These cytokines are collaboratively inhibitory for differentiation and effector functions of the reciprocal phenotypes; thus, a strong Th 2-oriented response tends to downregulate Th 1-type responses and vice versa (Raghupathy, 1997). However, a full-term pregnancy is distinguished by the expression of Th 2-type cytokines at the foetal-maternal interface (Suguitan *et al.* 2003). The significance of these cytokines in pregnancy include:

Tumor Necrosis Factor –alpha (TNF-α) - Higher production of TNF-α has been related to the risk for recurrent foetal loss, hypertensive syndromes, foetal growth restriction and gestational diabetes mellitus (Moreli *et al.* 2012). Hence, the regulation of TNF-α expression is vital for normal development of the placenta and its function (Gambling *et al*. 2002).

Interferon gamma (IFN-γ) – This is a pro-inflammatory cytokine (PIC) produced in the uterus at the early stage of parturition. IFN-γ have the distinct function of promoting angiogenesis in pregnancy that develops in conceptus-triggered locations to improve the success rate of pregnancy. However, complications such as foetal loss have been associated with increase in IFN-γ. In normal pregnancy, there is a decrease in IFN-γ secretion the clones of T cells in the decidua relative to systemic circulation (Lombardelli *et al.* 2016).

Table 2.1: Functions of cytokines

Cell type	Functions
T helper 1 (Th1)	Induce several cytotoxic and inflammatory reactions mediated by IL-2, IFN-γ, TNF-β and IL-12 and are widely responsible for cell-mediated inflammatory reactions, delayed-type hypersensitivity and tissue injury in infectious and autoimmune diseases.
Th2	Produce IL-4, IL-5, IL-6, IL-9 and IL-10 and are linked with help for antibody production by B-cells
T cytotoxic 1 (Tc1)	Secretes a Th1 pattern of cytokines
Tc2	Secretes a Th2 pattern of cytokines

Raghupathy (1997)

Interleukin 1 alpha (IL-1α) – IL-1 system was found to be crucial beyond 24 weeks' gestation in human cervico-vaginal fluid. However, IL-1α and IL-1β remained unchanged in late pregnancy (Heng *et al.* 2014). A study that assessed IL-1α in rabbit pregnancy showed the likelihood of a crucial role in blastocyst implantation (Yang *et al.* 1995).

Interleukin-2 – A study that evaluated IL-2 in amniotic fluid reported that it may likely be involved during early gestation and possibly labour (Zicari *et al.* 1995).

Interleukin-4 – Higher concentrations of IL-4 was secreted by decidua CD4 T cell clones compared to peripheral blood T cell clones (Lombardelli *et al.* 2016). A study of pregnancy in bitches showed elevation of this cytokine in early gestation (Pantaleo *et al.* 2013).

Interleukin-10 – This is an immunomodulatory cytokine produced by Th 2 cells (Holmes *et al.* 2003). This cytokine is very crucial for pregnancy maintenance and development. IL-10 levels in plasma was elevated in gravid women and mothers at post-delivery relative to the non-pregnant women (Holmes *et al.* 2003). IL-10 is involved in placental formation amidst other functions. It's immunosuppressive action also plays a major part in setting the balance of pro- and anti-inflammatory signs that are instrumental to the proper development of pregnancy (Moreli *et al.* 2012). Its production in early stage of pregnancy has also been reported in bitches (Pantaleo *et al.* 2013).

Interleukin-12 (p70) – found to be elevated in early and mid-stages of parturition. This is a key cytokine involved in regulating Th1/Th2 balance. The concentration of IL-12 (p70) was found to increase among preeclamptic women (Szarka *et al.* 2010).

Interleukin-17 – This cytokine is reported to be responsible for miscarriages. Serum IL-17 levels was elevated among pregnant women with preeclampsia (Molvarec *et al.* 2015). However, the combined secretion of IL-17 and IL-4 by fresh CD3+CD4+ T cells of the lining of the uterus is not deleterious to the success of pregnancy (Lombardelli *et al.* 2016).

Interleukin-18 – Increased circulation of IL-18 above IL-12 has been shown to favour Th-2 immunity required for a successful pregnancy (Szarka *et al.* 2010)

2.6.1 Changes in cytokine profile in pregnancy caused by single infections

Deficiencies in some of the immune modulatory molecules, antigen presentation by B-cells, macrophages and dendritic cells could initiate both Th 1 and Th 2 responses but the majority of particular cytokines in the local environment during activation of T-cell determines the shift in a Th 2 or Th 1 direction (Raghupathy, 1997). Dominance of Th 1 cytokines during pregnancy is linked to 'small for gestational age' babies, intrauterine growth retardation, recurrent spontaneous abortion and preterm childbirth (Moormann *et al.* 1999; Raghupathy *et al.* 2000).

Infection during pregnancy is an important factor for a Th 2 to Th 1 shift. Several researchers have evaluated changes in cytokine concentrations in systemic circulation and placental blood of gravid mothers with *P. falciparum* or HIV infections (Moormann *et al.* 1999; Suguitan *et al.* 2003; Bayoumi *et al.* 2008; Kfutwah *et al.* 2008; Sachdeva *et al.* 2008; Richardson and Weinberg, 2011). Quite a couple of works have associated cytokine expression to disease but results differ from one cohort population to another.

Malaria seemingly brings about a frail equilibrium of pro-inflammatory cytokines (PICs) and anti-inflammatory cytokines (AICs) (Iriemenam *et al.* 2009). TNF-α, a Th1 cytokine is believed to be involved in malaria pathogenesis particularly in cerebral malaria cases. In a region endemic for malaria in India, increased levels of IL-12, IL-5 and IL-6 distinguished severe malaria from mild malaria (Prakash *et al.* 2006). Hence, the antagonistic role of Th 1 and Th 2 immune responses to malaria infection determine the outcome of infection whether it will be protective or pathologic. Th 1 cytokines are vital in managing early parasitemia and this need to be counterbalanced later by a Th 2 response to initiate antibody secretion (Angulo and Fresno, 2002). IFN-γ, another pro-inflammatory cytokine was reportedly higher among non-immune patients diagnosed with acute and severe malaria while IL-12 was reportedly lower among patients with severe malaria and higher among those with acute malaria. However, there was no relationship between peripheral parasite count and cytokine serum concentration (Wroczyńska *et al.* 2005). Another study on the role of PICs and AICs in the presentation of malaria in children reported that IL-10 and IFN-γ levels were significantly elevated in children infected with malaria relative to the uninfected group thus conferring an immunological protection against malaria (Iriemenam *et al.* 2009).

Maternal white blood cells (WBCs) are needed in regulating malaria parasite density but they can also influence the production of uncontrolled inflammatory cytokines that are deleterious to the growing foetus. For instance, high levels of TNF–α and regulatory IL-10 may exacerbate anaemia in pregnancy (Suguitan *et al.* 2003). Another study reported a rise in the PICs' levels IFN-γ and TNF–α, in the placentas of malaria positive women in Malawi (Moormann *et al.* 1999). This increase in TNF–α was said to be associated with intrauterine growth retardation (IUGR) with similar findings reported in Cameroonian women. There was increase in release of cytokines such as TNF-α and IL-10 in *P. falciparum* infected placentae of women with pre-term births (Suguitan *et al.* 2003). The similarities in IFN-γ, TNF–α, IL-4 and IL-10 levels among full-term and pre-term delivery placentas in the study established that PTD had no association with changes in cytokine profiles. However, it was earlier reported that TNF-α may result in abortion due to uterine contraction or may cause foetal death (Raghupathy, 1997). In Nigeria, the shift towards a Th 1 response has been reported to be linked to increase in parasite density (Achidi *et al.* 2007; Nmorsi *et al.* 2010a). Thus, malaria impairs the normal immunological profile resulting in more complications in pregnancy.

Helminth infections on the contrary, display a strong polarization towards a Th 2 response (Abdoli and Pirestani, 2014) as IL-10 and/or TGF-β facilitate the long term survival of worms within the host resulting in an anti-inflammatory environment (Maizels *et al.* 2009). The development of strong survival strategies which includes the helminth's potential to exploit the immune system of the host facilitates their long-term survival in such hosts (Helmby, 2009). In addition to Th 2 cells, IL-9-producing T helper subset (Th 9) has been identified to be involved in immunological responses to helminths (Veldhoen *et al.* 2008). They can be derived from Th 2 cells or made de novo depending on the cytokine milieu. They partly mediate resistance in murine model of *Trichuris* infection (Veldhoen *et al.* 2008). Another set of T helper cells are the IL-17 secreting Th17 cells, which are active in inflammatory and autoimmune reactions. They are also identified in the pathology of some helminth infections (Rutitzky *et al.* 2005). In spite of the successful production of the Th2 response, helminths often have an extended period of survival in the host causing chronic infections. This long-term survival is aided by the induction of immunoregulatory mechanisms that aids in production of cytokines such as IL-10 and/or TGFβ thus

promoting an anti-inflammatory milieu (Maizels *et al.* 2009). This hinders the expulsion of the worms from the host but also protects against uncontrolled inflammation.

Pregnancy and HIV-1 infection jointly alters the immunological balance. While HIV-1 infection causes inflammatory responses and activation of lymphocytes, pregnancy also favours activation of Th 2 responses (Sachdeva *et al.* 2008). Some cytokines including TNF-α, IL-1α, IL-6 and IL-8 promote HIV-1 multiplication while IFN-ɣ and IFN-α inhibit its replication (Alfano and Poli, 2005). Decrease in IL-2 expression with a rise in Th2 cytokines in normal parturition reportedly favours pathogenesis of HIV-1 (Sutton *et al.* 2004). In a research work by Sachdeva and his co-workers, levels of IL-1α, IL-1β, IL-10 and IFN-γ were below the detection limit in a greater number of the recruited women, while there were no appreciable changes in concentrations of IL-2, IL-4 and IL-8 among the different groups (Sachdeva *et al.* 2008). Furthermore, pregnant women with HIV-1 infection had considerably elevated concentrations of TNF-α and also had increased levels of plasma TNF-α indicating that pregnancy elevates Th 1 responses in HIV-1 infection, thereby elevating the likelihood of prenatal transmission of HIV-1 (Sachdeva *et al.* 2008). Pro-inflammatory cytokines are vital in control and pathogenesis of HIV infection. For example, IL-2 and IFN-α control viral replication. HIV also causes a decreased production of the pro-inflammatory cytokines IL-12 and IFN-α and increased expression of IL-10 (Yadav *et al.* 2009). Increased IL-10 is involved in the impaired innate immune response observed in AIDS patients as well as loss of adaptive immunity (Ma and Montaner, 2000). In viral infections, IFN-γ activates macrophages to destroy the virus and also natural killer (NK) cells to destroy infected cells through release of granzyme and perforin (Machado *et al.* 2004). Macrophages as well as other antigen-presenting cells at the initial phase produce IL12 to stimulate NK cells to exert cytotoxicity and produce more IFN-γ (Machado *et al.* 2004).

2.6.2 Cytokine profile in response to co-infection: beneficial or detrimental

The immunoregulatory role of helminths has become an interesting area of research. The helminth-induced immunomodulation has been shown to have profound effect in co-infection and vaccine efficacy that could be beneficial or detrimental (Helmby and Bickle, 2006). Most helminth infections are associated with IL-10 and TGF-β cytokines playing regulatory roles. A key cytokine involved in immunological response to helminth infection is IL-10 and is important in the control of inflammation (Helmby and Bickle, 2006). The response of host to helminth infection elicits a Th 2 response causing significant production of IL-4, IL-5, IL-9, IL-10 and IL-13 (Kamal and Khalifa, 2006).

Studies on the pathogenesis and immunological interplay of malaria and helminth infections have also contributed greatly to a better awareness of the interrelationship between malaria and helminth parasites with the human host at the cellular level. However, since the immune system requires a balance between pro-inflammatory and anti-inflammatory responses to effectively combat concurrent infection with multiple pathogens, it is possible that the immune regulation could alter the progression of disease. Therefore, much interest has been generated on the likely role of helminths on the function of the immune system. It is suggested that the low rate of helminth infections in developed nations may be linked to higher occurrences of allergic and autoimmune disorders, while the high rates of these helminths in developing nations may negatively affect immune responses to concurrent infectious diseases, such as malaria. In an experiment performed in mice, it was proposed that co-infection of helminths and *P. berghei* may reduce the likelihood of cerebral malaria. Similar finding was reported in co-infection of larvae of *Brugia pahangi* and malaria parasites in CBA/J mice (Yan *et al.* 1997) while another study of co-infection with *H. polygyrus* had no impact on progression to cerebral malaria in mice (de Souza and Helmby, 2008).

In literature, the role of helminths infection in immune responses to other infectious diseases has been conflicting. According to a review, these differences in findings could be influenced by helminth species, sites of helminth infection, stage and/or intensity of infection (Helmby and Bickle, 2006). In another review article, the interaction between helminths and HIV showed that individuals with co-infection of HIV and schistosomes, a trematode had impaired immune responses to tetanus toxoid

vaccine. Infected persons mounted a Th 2 response while Th 1 or Th0 response was found among uninfected persons (Sabin *et al.* 1996). However, few reports exist on the interaction of HIV and intestinal helminths (nematodes) (Kamal and Khalifa, 2006). One of the reports was that helminth infection increased the probability of HIV infection in children born to infected mothers (Gallagher *et al.* 2005).

HIV and *P. falciparum* both induce an inflammatory response from immune cells, functionally impairing the dendritic cells (Yadav *et al.* 2009). This may also lead to higher occurrences of clinical malaria observed in cohort studies.

In Nigeria, some studies have focused on the prevalence of anaemia and also the epidemiology of single infections of malaria, intestinal helminths or HIV among expectant mothers but studies on co-infection of these pathogens are limited. Furthermore, there are no detailed evaluation of the systemic cytokine concentrations and iron status in single and co-infection of these diseases among pregnant women in this setting. Additional information on the immunological interplay of these diseases and the influence on host parameters would be beneficial in vaccine development.

CHAPTER THREE

METHODOLOGY

3.1 STUDY AREA

The Study Area for this research is Ibadan, a city in Southwest Nigeria. Ibadan is a semi-urban city located on latitude 7.3775° and longitude 3.9059° with an altitude of 181m above sea level. It is located 128 km inland northeast of Lagos state and 530 km southwest of Abuja. The city's total area is 3,080 km^2 and it has a tropical climate with two rainy seasons and almost constant temperatures throughout the year. It is the largest city by geographical area and the third most populous city in Nigeria with a population of 2.88 million. The major inhabitants are the Yorubas. There are eleven local governments in the metropolis consisting of five urban local governments (Ibadan North, Ibadan North-East, Ibadan North-West, Ibadan South-East and Ibadan South-West) and six semi-urban local governments (Akinyele, Egbeda, Ido, Lagelu, Ona Ara and Oluyole).

Two health facilities were selected for this study namely Adeoyo Maternity Hospital and Agbongbon Primary Healthcare Centre. Adeoyo Maternity Hospital is located in Ibadan North local government and it is a public secondary health facility providing maternal and child healthcare services for residents in Ibadan and its environs. Pregnant women registering for antenatal care for the first time were booked on a particular day in a week. The average number of women booking for ante-natal care monthly is about 480 pregnant women.

Agbongbon Primary Healthcare Centre is also a public health facility and is one of the operational 270 Primary Healthcare Centres (PHCs) owned by the Oyo State government. It is situated in Ibadan South-East local government and serves the local inhabitants who are predominantly petty traders and home makers. The average attendance on the weekly booking day is about 25 pregnant women.

3.2 STUDY DESIGN

This is a hospital-based cross-sectional study with inclusion and exclusion criteria as follows:

Inclusion criteria: Pregnant women between 18-45 years old who gave their consent were recruited on booking days (first time) after testing negative for HIV during routine screening in the ante-natal clinics. Confirmed HIV positive pregnant women were recruited from PEPFAR clinic.

Exclusion criteria: Those who didn't give their consent were not enrolled. Gravid women with observable complications were also excluded. Furthermore, patients that did not provide both blood and stool samples were not recruited.

3.2.1 Sample Size

This was calculated using the formula below at Z=1.96, P for expected prevalence rate and a precision (d) of 0.05 (Naing *et al.* 2006).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Prevalence values for each of the infections were based on previous reports from studies among pregnant women in the study area at the time of recruitment. Prevalence values of 48% was used for malaria (Ayoola *et al.* 2011), 43.4% for intestinal helminths (Alli *et al.* 2011) and 2.9% for HIV (Adesina *et al.* 2011).

Malaria:

$$n = \frac{Z^2 P (1-P)}{d^2}$$

$$= \frac{(1.96)^2 \times 0.48 (1-0.48)}{(0.05)^2} = 384$$

Helminths:

$$n = \frac{Z^2 P (1-P)}{d^2}$$

$$= \frac{(1.96)^2 \times 0.43 (1-0.43)}{(0.05)^2} = 377$$

HIV:

$$n = \frac{Z^2 P (1-P)}{d^2}$$

$$= \frac{(1.96)^2 \times 0.029 (1-0.029)}{(0.05)^2} = 43$$

Using the highest sample size, a minimum of 422 pregnant women were proposed to be recruited having added 10% of the calculated value to suffice for unexpected outcomes.

3.3 ENROLMENT/SELECTION OF STUDY PARTICIPANTS

Pregnant women in the first, second or third trimesters reporting for antenatal care for the first time were enrolled from the selected hospitals while confirmed HIV infected pregnant women were recruited from Presidential Emergency Plan for AIDS Relief (PEPFAR) clinic, Adeoyo Maternity Hospital, Yemetu, Ibadan, Oyo State, Nigeria. One thousand and seventy five (1,075) parturient women consented to participate in the research and were screened for malaria parasitemia. The 368 women who did not meet up with the inclusion criteria were excluded from the study. Overall, 707 pregnant women were recruited. They comprised of 490 human immunodeficiency virus (HIV) negative expectant mothers enrolled from the ante-natal clinics (ANCs) and 217 HIV positive from the HIV clinic.

3.4 SAMPLE COLLECTION

Personal details, blood and stool samples were obtained from pregnant women who consented to participate in the study. Samples were collected from August 2011 to March 2015. Five millilitres of whole blood was collected through venepuncture into plain/serum bottles for all patients while another 5ml of whole blood was withdrawn into vacutainer tubes for HIV patients. Patients were given a universal bottle and a wooden spatula for stool collection. Patients' specimen were collected at the hospitals and the blood samples were processed at Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan. The serum obtained after centrifugation of whole blood was stored at -20°C until required for analysis.

3.4.1 Administration of Questionnaires

Pretested well-structured close-ended questionnaires were administered by trained data collectors in the native language of the subjects (Yoruba) as majority of them were semi-literate. Demographic details including age, parity, gestational age, use of malaria prophylaxis within two weeks prior to recruitment and the use of antihelminthic drugs within six months prior to recruitment were obtained from the women. Antiretroviral drug usage was also obtained from the HIV-infected women. The gestational age was calculated from the date of the last menstrual period. Presence of fever was determined by axillary temperature ≥ 37.5°C. The socio-economic status of the women were assessed using the following parameters; level of education, occupation, type of accommodation, type of wall and toilet facility in the accommodation, source of portable water in the accommodation, presence of screens on the windows or doors, presence of stagnant water and open drainage system around the house. Other indices of assessment were covering of water collection containers, frequency of hand wash, habit of walking barefoot, common place of defaecation and exposure to health education. In assessment of occupation, variables were grouped as unemployed/student; petty traders; primary school teachers, junior civil servants and artisans; high school teachers, middle level civil servants and middle business persons; and major business persons and professionals.

3.5 MALARIA MICROSCOPY AND ESTIMATION OF PARASITE DENSITY

Slides were processed and viewed at Drug Research Unit and Home Management Lab, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan. Thick blood smears were made on labelled glass slides, dried and stained with freshly prepared 10% working solution of Giemsa stain for 10 min. The slides were rinsed in slow running water and then allowed to dry. The slides with immersion oil were viewed under x100 objective using light microscope. *P. falciparum* were counted in each field against 200-500 white blood cells (WHO, 2010) and the parasite density was estimated thus:

$$\text{Parasite density } (\mu L) = \frac{\text{No of asexual stages of malaria parasites counted} \times 8000}{\text{Number of leukocytes counted}}$$

3.6 STOOL PREPARATION AND HELMINTH QUANTIFICATION

Analysis of stool samples was carried out at Department of Microbiology and Parasitology, College of Medicine, University of Ibadan. Helminths in stools were identified and quantified using wet preparation Kato-Katz methods respectively.

Wet preparation method

An estimate of 1 gram each of freshly collected faeces was placed on two ends of a glass slide with a drop of normal saline or iodine added respectively. The two ends were covered with cover slips and viewed under light microscope for presence of helminth eggs (WHO, 1994).

Kato-Katz method

The template of the Kato-Katz kit was placed on a glass slide. The hole of the template was then filled with the sieved stool sample. The template was removed and the stool covered firmly with cellophane that had been pre-soaked in glycerol with malachite green. It was then viewed under the light microscope. The egg count of each identified parasite was multiplied by 24 to obtain the egg count per gram (WHO, 1994). The egg count per gram (epg) was classified as: Low intensity (*A. lumbricoides* 1- 4999; *T. trichiura* 1-999; hookworms 1-1999), moderate intensity (*A. lumbricoides* 5000-49,999; *T. trichiura* 1000-9999; hookworms 2000-3999) and heavy intensity infection (*A. lumbricoides* ≥ 50,000; *T. trichiura* ≥ 10,000; hookworms ≥ 4000) (WHO, 1999).

3.7 GROUPING OF PARTICIPANTS

After collection and testing of patients' samples, they were grouped as follows:

Group 1 – *P. falciparum* infection only (PF)

Group 2 – Helminth infection only (H)

Group 3 – HIV infection only (HIV)

Group 4 – *Plasmodium falciparum* and Helminth co-infection (PF+H)

Group 5 – Helminth and HIV co-infection (H+HIV)

Group 6 – *P. falciparum* and HIV combined infections (PF+HIV)

Group 7 – *P. falciparum*, Helminth and HIV combined infections (PF+H+HIV)

Group 8 – Uninfected group (N)

3.8 ABSOLUTE CD4 COUNT ESTIMATION

Absolute CD4 count estimation of blood samples of HIV patients was done using the flow cytometer. The principle of flow cytometry is based on the measurement of multiple morphological features of a single cell such as size and granularity simultaneously as the cell flows in suspension through a measuring device. The fluorescence of the cells being probed is from dyes or monoclonal antibodies to extracellular or intracellular molecules of the cell tagged to fluorescent dyes. Afterwards, the cell population of interest can be identified and gated (Adan *et al.* 2017).

3.9 HIV RNA QUANTIFICATION FOR VIRAL LOAD DETERMINATION

This procedure was carried out using Polymerase Chain Reaction. This allows for the amplification of specific DNA fragment from a complex pool of DNA using a DNA-polymerase enzyme that is involved in the replication of the cellular genetic material. It synthesizes a complementary sequence of DNA when a primer is connected to one of the DNA strands in the specific site chosen to start the synthesis. This limits the sequence to be replicated and the result is the amplification of a particular DNA sequence with billions of copies (Valones *et al.* 2009).

3.10 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETERMINATION OF CONCENTRATIONS OF FERRITIN, TRANSFERRIN, CYTOKINES AND VIRAL LOAD

Concentrations of ferritin, transferrin, the cytokines and viral load were determined using ELISA as described by Gan and his colleague (Gan and Patel, 2013). ELISA is used for the detection and quantification of specific antigens or antibodies in a given sample. It uses the basic immunology principle of an antigen binding to its specific antibody thus allowing the detection of minute quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample. The most commonly used enzymes for detection are alkaline phosphatase and glucose oxidase. The antigen is allowed to bind to a specific antibody, which is itself subsequently detected by a secondary, enzyme-coupled antibody. A chromogenic substrate for the enzyme yields a visible

colour change or fluorescence, indicating the presence of antigen and the amount of antibody or antigen in the sample. These assays were performed at Immunology Lab, Chemical Pathology Department, College of Medicine, University of Ibadan.

3.11 PACKED CELL VOLUME DETERMINATION

This procedure was carried out at Drug Research Unit, IAMRAT, College of Medicine, University of Ibadan. Blood obtained through finger prick was withdrawn into capillary tube and sealed at one end with plasticine. The capillary tubes were then spun using the Hawksley microcentrifuge (Hawksley Ltd, Lancing, UK) at 3000g for 10 min. The result was obtained using the haematocrit reader. Anaemia in pregnancy was defined as PCV < 33% and was further classified as Mild – 30-32%, Moderate – 21-29% and Severe - < 21% (WHO, 1999).

3.12 SERUM IRON DETERMINATION

Samples were processed using Atomic Absorption Spectrophotometry as described by Beaty and Kerber (Beaty and Kerber, 1993). The principle is based on that elements in the gas phase absorb light at very specific wavelengths. The liquid is drawn into a flame where it is ionised in the gas phase. Light of a specific wavelength appropriate to the element being analysed is shone through the flame. The absorption is proportional to the concentration of the element. Normal serum iron values ranged 0.5-1.5mg/L (Perkin-Elmer Corporation, 1996).

3.13 ETHICAL CONSIDERATION

Consent was obtained from the pregnant women and those who were not willing to participate were excluded from the study. Ethical approval for this study was obtained from UI/UCH Ethical Review Committee, College of Medicine, University of Ibadan (UI/EC/10/0180) and Oyo State Ministry of Health.

3.14 DATA ANALYSIS

SPSS version 22.0 (IBM® SPSS®, Chicago, IL, USA) was used for the data analysis while Graph Pad Prism software 6.01 (GraphPad Software, San Diego, CA, USA) was used in plotting graphs. Pearson Chi-Square was used to test for associations while Student t-test was used to determine significant differences in mean values between two groups. For non-parametric data, significant differences in median values between two groups were determined using Mann-Whitney U test. Multiple logistic regression was used to determine risk factors for anaemia, *Plasmodium* and helminth infections. Correlations between parasite density and levels of parameters assessed for iron and anaemia was determined by Spearman's correlation coefficient. Significant level was set at $p<0.05$.

CHAPTER FOUR

RESULTS

4.1 PATIENTS' SOCIO-DEMOGRAPHIC DETAILS

The total number of enrolled pregnant women was 707 and 6.4% of these women presented during the first trimester of pregnancy. The mean age of the 707 recruited parturient women was 28.6±5.4 yrs old and their average weight and temperature were 64.3±12.1 kg and 36.5±0.8°C respectively. About half (48.0%) of the women were in the late gestational stage. They were mostly multigravidas (75.1%) and secondary school leavers (56.9%). The major occupation of the recruited women was petty trading (54.4%) and more than 70% of these women lived in rented houses. The major source of portable water was from deep wells with the use of bucket and rope and 5.4% of the women had no toilet in the house. Table 4.1 showed details of the socio-demographic status of the women.

Table 4.1: Socio-demographic characteristics of recruited pregnant women

Characteristics	N	Status	n (%)
Age	700	*< 20 years*	22 (3.1)
		20-34 years	564 (80.6)
		≥ 35 years	114 (16.3)
Trimester	658	*First*	42 (6.4)
		Second	300 (45.6)
		Third	316 (48.0)
Gravidity	695	*Primigravidae*	173 (24.9)
		Multigravidae	522 (75.1)
Level of education	703	*None*	5 (0.7)
		Primary/quranic	138 (19.6)
		Secondary	400 (56.9)
		Post-secondary	160 (22.8)
Occupation	702	*Student/Unemployed*	52 (7.4)
		Petty trader	382 (54.4)
		Primary school teacher/Junior civil servant/Artisan	188 (26.8)
		High school teacher/Middle level civil servant/Middle businessman	62 (8.8)
		Major business person/Professional	18 (2.6)
Type of accommodation	691	*Rented*	531 (76.8)
		Family owned	58 (8.4)
		Self	77 (11.1)
		Parent owned	25 (3.6)
Type of wall	705	*Unplastered mud wall*	8 (1.1)
		Plastered mud wall	27 (3.8)
		Unplastered cement block wall	51 (7.2)
		Cement wall plastered	619 (87.8)

Table 4.1 contd.: Socio-demographic characteristics of recruited pregnant women

Characteristics	N	Status	n (%)
Source of portable water	706	*None*	1 (0.1)
		Well with pump	40 (5.7)
		Well with bucket and rope	452 (64.0)
		Pipe-borne water	72 (10.2)
		Borehole	141 (20.0)
Toilet facility	705	*None*	38 (5.4)
		Pit latrine	315 (44.7)
		Water system	352 (49.9)

4.2 PATTERN AND STATUS OF INFECTION

Overall, there were 114 (16.1%) cases of *Plasmodium falciparum* infection and 62 (8.8%) cases of helminth infection (Table 4.2). Seven women were infected with more than one species of helminths. 67 (9.4%) had co-infections (*Plasmodium* and helminths – 1.1%, *Plasmodium* and HIV – 7.4%, HIV and helminths – 0.8%, *Plasmodium,* HIV and helminths – 0.1%) while 258 (36.4%) had single infections (*Plasmodium* – 7.5%, Helminth - 6.6%, HIV – 22.3%). A higher proportion (54%) of the enlisted women were uninfected.

In Table 4.3, the geometric mean malaria parasite density was lower among those with combined infections of *P. falciparum* and helminths (470/µL) in relation to those with *P. falciparum* only (1193/µL). The individual with combined infections of *Plasmodium,* helminths and HIV had the highest parasite density of 3582/µL. However, *Ascaris lumbricoides* egg count was highest among those with co-infection of *P. falciparum* and helminths (1959 eggs per gram, epg) and lowest among those with helminths and HIV co-infection (1028 epg). Among those infected with HIV, the CD4 count was lowest among those with combined infection of *P. falciparum* (294 cells/mm^3) and highest among those with combined infection of helminths. However, these differences were not significant. The viral load was significant across the groups with the highest value found among those infected with *P. falciparum* and HIV (Table 4.3).

Table 4.2: Prevalence of infection in the study population.

Characteristics (n=707)	Malaria	Helminths			HIV
	PF	AL	TT	HW	HIV
No infected	114	60	6	3	217
Prevalence (%)	16.1	8.4	0.8	0.4	30.7
Geometric mean parasite density	1184 parasites/µL of blood	1430epg	36epg	237epg	383 cells/mm^3

Key: PF- *P. falciparum,* AL- *A. lumbricoides*, TT - *T. trichiura*, HW – Hookworm, epg – eggs per gram

Table 4.3: Geometric mean count of parasite density, cell count and viral load among the infected groups.

		Malaria	Helminths			HIV	
	n	PF (parasites/μL of blood)	AL (epg)	TT (epg)	HW (epg)	CD4 (cells/mm^3)	Viral load
PF only	53	1193	-	-	-	-	-
H only	42	-	1414	36	237	-	-
HIV only	71	-	-	-	-	407	19,722
PF+H	8	470	1959	-	-	-	-
H+HIV	6	-	1028	-	-	416	27,327
PF+HIV	52	1327	-	-	-	294	104,221
PF+H+HIV	1	3582	1320	-	-	301	40,025
P-value		0.156	0.661	-	-	0.127	0.017*

Key: PF – *P. falciparum*, H – Helminth, AL – *A. lumbricoides*, TT – *T. trichiura*, HW – hookworm, epg – eggs per gram *significant at $\alpha_{0.05}$

Furthermore, in Table 4.4, the number of cases of malaria in relation to age, trimester and gravidity showed no significant association in these variables and *P. falciparum* infection. However, 85% (97/114) of those infected with *P. falciparum* were within 20-34 years old, 48.6% (53/109) were in second trimester and 77.2% (88/114) were multigravids. Assessment of the parasite density in relation to age, trimester and gravidity showed that the geometric mean parasite density was highest among those < 20 years old (7858/µL) and they had malaria parasite density ≥ 1000/µL . However, the associations were not statistically significant (Table 4.5). The geometric mean malaria parasite density was highest among those in third trimester (1233/µL) and primigravids (1673/µL) but these relationships were also not of statistical significance (Table 4.5).

The number of helminths in relation to age, trimester and gravidity was also determined. However, analysis was done for *Ascaris* only as other helminths found in this study were too low for statistical analysis. Table 4.4 showed that 86% (49/57) of those infected with *Ascaris* were 20-34 years old, 52.7% (29/55) were in second trimester and 73.7% (42/57) were multigravids. However, the difference in proportion was not significant. Similarly, there were no significant associations in egg count and age, trimester or gravidity but the geometric mean egg counts were highest in 20-34 years old (1444 eggs per gram), first trimester (2272 eggs per gram) and primigravids (1924 eggs per gram). About 77.2% (44/57) of those infected with *Ascaris* had egg counts < 5000 eggs per gram (Table 4.6).

In Table 4.4, the prevalence of HIV in relation to age, trimester and gravidity showed that significantly higher proportions of those infected with HIV were 20-35 years old (75.3%), in the third trimester (57.1%) and multigravids (91.9%). Assessment of the CD4 count in relation to age, trimester and gravidity showed that the geometric mean CD4 count was lowest among those ≥ 35 years (377 cells/mm^3), in second trimester (359 cells/mm^3) and multigravid (375 cells/mm^3). However, the associations were not statistically significant. Among those infected with HIV, 54.4% (49/90) had CD4 counts ≥ 350 cells/mm^3 (Table 4.7).

Table 4.4: Prevalence of infections by age, trimester and gravidity

Age group	n	PF (%)	AL (%)	HIV (%)
< 20 years	22	3 (2.6)	2 (3.5)	1 (0.5)
20-34 years	564	97 (85.1)	49 (86.0)	162 (75.3)
≥ 35 years	114	14 (12.3)	6 (10.5)	52 (24.2)
Total	700	114 (16.3)	57 (8.1)	215 (30.7)
χ^2		1.799	1.514	20.023
P-value		0.407	0.469	<0.0001*
Trimester				
First	42	6 (5.5)	4 (7.3)	11 (5.8)
Second	300	53 (48.6)	29 (52.7)	70 (37.0)
Third	316	50 (45.9)	22 (40.0)	108 (57.1)
Total	658	109 (16.6)	55 (8.4)	189 (28.7)
χ^2		0.547	1.549	8.980
P-value		0.761	0.461	0.011*
Gravidity				
Primigravid	173	26 (22.8)	15 (26.3)	17 (8.1)
Multigravid	522	88 (77.2)	42 (73.7)	192 (91.9)
Total	695	114 (16.4)	57 (8.2)	209 (30.1)
χ^2		0.317	0.067	44.895
P-value		0.573	0.795	<0.0001*

Key: PF - *P. falciparum,* AL- *A. lumbricoides* *significant at $\alpha_{0.05}$

NB: Egg counts of *T. trichiura* and hookworms were excluded from analysis because of the small sample size.

Table 4.5: Malaria parasite density in relation to age, trimester and gravidity

Age group		<1000/μL	≥ 1000/μL	Total	GM
< 20 years		--	3 (100%)	3 (100%)	7858
20-34 years		47 (48.5%)	50 (51.5%)	97 (100%)	1107
≥ 35 years		6 (42.9%)	8 (57.1%)	14 (100%)	1264
Total		53 (46.5%)	61 (53.5%)	114 (100%)	
χ^2	2.831				
P-value	0.243				0.779
Trimester					
First		4 (66.7%)	2 (33.3%)	6 (100%)	1174
Second		24 (45.3%)	29 (54.7%)	53 (100%)	1128
Third		22 (44%)	28 (56.0%)	50 (100%)	1233
Total		50 (45.9%)	59 (54.1%)	109 (100%)	
χ^2	1.123				
P-value	0.570				0.074
Gravidity					
Primigravid		10 (38.5%)	16 (61.5%)	26 (100%)	1673
Multigravid		43 (48.9%)	45 (51.1%)	88 (100%)	1070
Total		53 (46.5%)	61 (53.5%)	114 (100%)	
χ^2	0.873				
P-value	0.350				0.248

Key: GM – Geometric mean

Table 4.6: ***Ascaris*** **egg count in relation to age, trimester and gravidity**

Age group		<5000 epg	≥ 5000 epg	Total	GM
< 20 years		2 (100%)	--	2 (100%)	1364
20-34 years		38 (77.6%)	11 (22.4%)	49 (100%)	1444
≥ 35 years		4 (66.7%)	2 (33.3%)	6 (100%)	1340
Total		44 (77.2%)	13 (22.8%)	57 (100%)	
χ^2	0.972				
P-value	0.615				0.869
Trimester					
First		2 (50.0%)	2 (50.0%)	4 (100%)	2272
Second		24 (82.8%)	5 (17.2%)	29 (100%)	1299
Third		16 (72.7%)	6 (27.3%)	22 (100%)	1489
Total		42 (76.4%)	13 (23.6%)	55 (100%)	
χ^2	2.359				
P-value	0.308				0.378
Gravidity					
Primigravid		12 (80.0%)	3 (20.0%)	15 (100%)	1924
Multigravid		32 (76.2%)	10 (23.8%)	42 (100%)	1286
Total		44 (77.2%)	13 (22.8%)	57 (100%)	
χ^2	0.091				
P-value	0.763				0.489

Key: GM – Geometric mean

Table 4.7: CD4 count in relation to age, trimester and gravidity of HIV-infected pregnant women.

Age group		< 350 cells/mm^3	≥ 350 cells/mm^3	Total	GM
< 20 years		--	1 (100%)	1 (100%)	608
20-34 years		32 (46.4%)	37 (53.6%)	69 (100%)	379
≥ 35 years		9 (45.0%)	11 (55.0%)	20 (100%)	377
Total		41 (45.6%)	49 (54.4%)	90 (100%)	
χ^2	0.858				
P-value	0.651				0.522
Trimester					
First		3 (60.0%)	2 (40.0%)	5 (100%)	440
Second		14 (51.9%)	13 (48.1%)	27 (100%)	359
Third		23 (41.8%)	32 (58.2%)	55 (100%)	383
Total		40 (46.0%)	47 (54.0%)	87 (100%)	
χ^2	1.154				
P-value	0.562				0.458
Gravidity					
Primigravid		2 (22.2%)	7 (77.8%)	9 (100%)	474
Multigravid		38 (46.9%)	43 (53.1%)	81 (100%)	375
Total		40 (44.4%)	50 (55.6%)	90 (100%)	
χ^2	2.000				
P-value	0.157				0.114

Key: GM – Geometric mean

In Table 4.8, 77.2% (44/50) of those infected with helminths had egg count of <5000 eggs per gram (epg). Similarly, 71.4% (5/7) of those with co-infection of *Ascaris* and HIV had egg count of <5000 epg but this relationship was not statistically significant (p=0.698). Among those with co-infection of *Ascaris* and *Plasmodium*, 77.8% (7/9) had egg count of <5000 epg. This was also not statistically significant (p=0.964).

Furthermore, 53.5% (61/114) of those infected with *Plasmodium* had malaria parasite count ≥1000/µL. Similarly, 60.4% (32/53) of those with co-infection of *Plasmodium* and HIV had parasite density ≥1000/µL but this association was not statistically significant (p=0.171). A greater percentage of those with co-infection of *Ascaris* and *Plasmodium* (55.5%) also had parasite density ≥1000/µL. This was also not statistically significant (p=0.898) (Table 4.8).

About 54.9% (50/91) of those with HIV had CD4 count ≥ 350 cells/mm^3. However, 58.8% (10/17) of those with co-infection of *Plasmodium* and HIV had CD4 count <350 cells/mm^3 but this association was not statistically significant (p=0.698). In co-infection of *Ascaris* and HIV, an equal proportion (50%) had either a CD4 count <350 cells/mm^3 or a CD4 count ≥350 cells/mm^3 (Table 4.8).

Table 4.8: Association between co-infection and intensity of infection.

	Ascaris egg count			χ^2	*P*-value
	<5000 epg	≥5000 epg	Total		
HIV positive	5 (71.4%)	2 (28.6%)	7 (100%)		
HIV negative	39 (78%)	11 (22%)	50 (100%)		
Total	44 (77.2%)	13 (22.8%)	57 (100%)	0.151	0.698
PF positive	7 (77.8%)	2 (22.2%)	9 (100%)		
PF negative	37 (77.1%)	11 (22.9%)	48 (100%)		
Total	44 (77.2%)	13 (22.8%)	57 (100%)	0.002	0.964
	Malaria parasitemia count				
	<1000/µL	≥1000/µL	Total		
HIV positive	21 (39.6%)	32 (60.4%)	53 (100%)		
HIV negative	32 (52.5%)	29 (47.5%)	61 (100%)		
Total	53 (46.5%)	61 (53.5%)	114 (100%)	1.878	0.171
Ascaris positive	4 (44.4%)	5 (55.5%)	9 (100%)		
Ascaris negative	49 (46.7%)	56 (53.3%)	105 (100%)		
Total	53 (46.5%)	61 (53.5%)	114 (100%)	0.016	0.898
	CD4 count				
	<350 cells/mm^3	≥350 cells /mm^3	Total		
PF positive	10 (58.8%)	7 (41.2%)	17 (100%)		
PF negative	31 (41.9%)	43 (58.1%)	74 (100%)		
Total	41 (45.1%)	50 (54.9%)	91 (100%)	1.601	0.206
	<350 cells/mm^3	≥350 cells /mm^3	Total		
Ascaris positive	2 (50.0%)	2 (50.0%)	4 (100%)		
Ascaris negative	39 (44.8%)	48 (55.2%)	87 (100%)		
Total	41 (45.1%)	50 (54.9%)	91 (100%)	0.041	0.839

Key: PF – *P. falciparum*

NB: Egg counts of *T. trichiura* and hookworms were excluded from analysis because of the small sample size.

4.3 RISK FACTORS FOR SUSCEPTIBILITY TO MALARIA PARASITEMIA AND HELMINTH INFECTIONS.

As shown in Table 4.9, the only variable showing a significant prediction of the likelihood of harbouring malaria parasitemia is HIV infection. Malaria parasitemia is 2.3 times more likely among those infected with HIV (OR=2.27, CI=1.51-3.42). Other variables showed no significant risk of malaria of infection.

In relation to helminth, univariate logistic regression showed that residents of houses with pit latrines or no toilet facilities, common defaecation in pit latrines or potties and those without HIV were more likely to harbour helminths. HIV infection was less likely to occur among those infected with helminths as shown in Table 4.10 (OR=0.26, CI=0.12-0.59). Residents of houses with no toilet facility or pit latrine were 4.5 times and 2.7 times more likely to be infected with helminths compared with those without helminth infection respectively. Based on their common place of defaecation, the use of pit latrine and potty posed greater risks of helminth infection. Other variables were not identified as risk factors for helminthiasis (Table 4.10).

Table 4.9: Univariate logistic regression predicting the occurrence of malaria parasitemia

Variables	Reference Category	Crude Odds Ratio (OR)	95% CI	*P*-value
Age (20-34 years)	Age (<20 years)	1.13	0.38-4.53	0.664
Age (≥ 35 years)	"	0.89	0.23-3.39	0.860
Second trimester	First trimester	1.29	0.52-3.21	0.588
Third trimester	"	1.13	0.45-2.82	0.797
Primigravid	Multigravid	0.87	0.54-1.40	0.574
Mud wall unplastered	Cement wall plastered	0.76	0.09-6.24	0.798
Mud wall plastered	"	1.86	0.77-4.52	0.170
Cement wall unplastered	"	0.99	0.45-2.17	0.978
No window/door screen	Window/door screen	1.26	0.63-2.51	0.514
Stagnant water	No stagnant water	0.67	0.41-1.08	0.096
Open drainage	No open drainage	0.93	0.59-1.46	0.751
A.lumbricoides	No *Ascaris*	0.91	0.44-1.91	0.805
Helminths generally	No helminths	0.87	0.42-1.83	0.719
Ascaris egg (<5000 epg)	*Ascaris* egg (>5000 epg)	1.04	0.19-5.75	0.964
HIV	No HIV	2.27	1.51-3.42	<0.0001*
CD4 count (<350)	CD4 (>350)	1.98	0.68-5.78	0.211

*significant at $\alpha_{0.05}$ epg – eggs per gram

Table 4.10: Univariate logistic regression predicting the occurrence of helminth infection

Variables	Reference Category	Crude Odds Ratio (OR)	95% CI	*P*-value
Age (20-34 yrs)	Age (<20 years)	0.64	0.18-2.25	0.489
Age (≥ 35 yrs)	“	0.41	0.10-1.75	0.230
Second trimester	First trimester	1.13	0.38-3.39	0.821
Third trimester	“	0.78	0.26-2.37	0.663
Primigravid	Multigravid	1.16	0.64-2.08	0.630
Walking barefoot	No walking barefoot	1.34	0.79-2.26	0.279
P. falciparum	No *P. falciparum*	0.87	0.42-1.83	0.719
MP count ≥1000	MP <1000	1.09	0.28-4.30	0.898
HIV	No HIV	0.26	0.12-0.59	0.001*
CD4 count (<350)	CD4 (>350)	1.23	0.17-9.14	0.839
No toilet	Water system	4.45	1.71-11.55	0.002*
Pit latrine	“	2.70	1.49-4.90	0.001*
Place of common defaecation				
Open field	Water system	3.80	0.99-14.63	0.052
Dunghill	“	7.18	0.70-73.83	0.097
Pit latrine	“	3.15	1.58-6.28	0.001*
Potty	“	2.87	1.37-6.03	0.005*

Key: MP – Malaria parasite *****significant at $p<0.05$

4.4 ANAEMIA IN RELATION TO INFECTION STATUS

The mean packed cell volume (PCV) of the recruited women was 31.7±5.4% and 387 (57.6%) of the pregnant women were anaemic (mild – 24.0%, moderate – 32.0%, severe – 1.6%). A higher proportion of those with anaemia had moderate anaemia (21%-28%). These were expectant mothers with helminths, HIV, combined infections of *Plasmodium* and HIV and the uninfected. More than 50% cases of severe anaemia were those with HIV only (Table 4.11).

Relative to the mean PCV of the uninfected group (32.9 ± 5.6%), PCV was lower in all the infected groups and was highly significant among those with co-infection of *Plasmodium* and HIV (29.7 ± 4.8%) and those with single infections of HIV (29.3 ± 5.0%) and *Plasmodium* (31.0 ± 4.0%) relative to the uninfected as shown in Table 4.11. Helminth infection further reduced the mean PCV in co-infection with *Plasmodium* relative to the single infections though not statistically significant (Table 4.11). In co-infection of helminth with HIV, the mean PCV was elevated relative to helminth only and HIV only (Table 4.11). HIV also further reduced the PCV in co-infection with *Plasmodium* relative to *Plasmodium* infection only but the differences were not statistically significant (Table 4.11).

As shown in Table 4.12, 67.3% (74/110) of those with *Plasmodium* infection had PCV <33%. In relation to the parasite density, 71.2% (42/74) of those who had parasite density ≥1000/μL were anaemic but there was no significant association between them. Similarly, 60.3% (35/58) of those with helminth infection had PCV <33% but the proportion of those who had anaemia was not statistically significant from those without anaemia. About 61.5% (8/13) of those who had egg count of ≥5000 epg were anaemic but this was also not statistically significant (p=0.950). Furthermore, 75.6% (149/197) of those with HIV infection had PCV <33%. In relation to the CD4 count, 97.3% (36/37) of those with CD4 count <350 cells/mm^3 were anaemic (p=0.010).

Table 4.11: Mean PCV values and anaemia classification in relation to infection status.

Groups	n	Mean ± SD	% anaemia	Mild	Moderate	Severe
PF	53	31.0 ± 4.0[a]	60.4	16 (30.2%)	16 (30.2%)	--
H	44	32.2 ± 5.0	54.5	11 (25.0%)	13 (29.5%)	--
HIV	143	29.3 ± 5.0[b]	76.2	39 (27.3%)	64 (44.8%)	6 (4.2%)
PF+H	8	29.9 ± 4.7	75	3 (37.5%)	3 (37.5%)	--
H+HIV	5	30.6 ± 4.0	80	2 (40.0%)	2 (40.0%)	--
PF+HIV	48	29.7 ± 4.8[c]	72.9	15 (31.3%)	18 (37.5%)	2 (4.2%)
PF+H+HIV	1	27.0	100	--	1 (100%)	--
Uninfected	370	32.9 ± 5.6[a,b,c]	47.6	75 (20.3%)	98 (26.5%)	3 (0.8%)

$\chi^2 = 54.955$, $P<0.0001$

Key: PF – *P. falciparum*, H – Helminth, SD – standard deviation

Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.12: Association between anaemia and infections and the parasite densities.

Anaemia	PF positive	PF negative	Total	χ^2	*P*-value
Present	74 (67.3%)	313 (55.7%)	387 (57.6%)		
Absent	36 (32.7%)	249 (44.3%)	285 (42.4%)		
Total	110 (100%)	562 (100%)	672 (100%)	5.050	0.025*
	Malaria parasitemia count				
	< 1000/µL	≥ 1000/µL			
Present	32 (62.7%)	42 (71.2%)	74 (67.3%)		
Absent	19 (37.3%)	17 (28.8%)	36 (32.7%)		
Total	51 (100%)	59 (100%)	110 (100%)	0.885	0.347
	H positive	H negative	Total		
Present	35 (60.3%)	352 (57.3%)	387 (57.6%)		
Absent	23 (39.7%)	262 (42.7%)	285 (42.4%)		
Total	58 (100%)	614 (100%)	672 (100%)	0.197	0.657
	Ascaris egg count				
	< 5000 epg	≥ 5000 epg	Total		
Present	25 (62.5%)	8 (61.5%)	33 (62.3%)		
Absent	15 (37.5%)	5 (38.5%)	20 (37.7%)		
Total	40 (100%)	13 (100%)	53 (100%)	0.004	0.950
	HIV positive	HIV negative	Total		
Present	149 (75.6%)	238 (50.1%)	387 (57.6%)		
Absent	48 (24.4%)	237 (49.9%)	285 (42.4%)		
Total	197 (100%)	475 (100%)	672 (100%)	37.158	<0.0001*
	CD4 count				
	< 350	≥ 350	Total		
Present	36 (97.3%)	35 (77.8%)	71 (86.6%)		
Absent	1 (2.7%)	10 (22.2%)	11 (13.4%)		
Total	37 (100%)	45 (100%)	82 (100%)	6.661	0.010*

Key: PF – *P. falciparum*, H – Helminth *significant at $\alpha_{0.05}$

Univariate logistic regression demonstrated that *P. falciparum* and HIV infections were predisposing factors for anaemia. However, helminth as a predisposing factor for anaemia was not significant (Table 4.13). Similarly, the odds of being anaemic was 2.1 times higher among those with co-infection of *Plasmodium* and HIV (OR=2.08, CI=1.08-4.01). In addition, the odds of being anaemic was 10.3 times higher among those with CD4 count <350 cells/mm^3. Other variables showed no significant risk of anaemia (Table 4.13).

Table 4.13: Univariate logistic regression predicting the occurrence of anaemia

Variables	Odds Ratio (OR)	95% CI	*P*-value
P. falciparum	1.64	1.06-2.52	0.026*
MP count (<1000/μL)	0.68	0.31-1.52	0.348
P. falciparum + HIV	2.08	1.08-4.01	0.029*
HIV	3.09	2.13-4.48	<0.0001*
CD4 count (<350 cells/mm^3)	10.29	1.25-84.64	0.030*
Helminths generally	1.13	0.65-1.96	0.657
A.lumbricoides	1.15	0.66-2.02	0.621
Ascaris egg count (< 5000 epg)	1.04	0.29-3.78	0.950
P. falciparum + helminth	2.23	0.45-11.12	0.329
Helminths + HIV	2.97	0.33-26.68	0.332
First trimester	1.18	0.59-2.37	0.646
Second trimester	1.20	0.87-1.67	0.268
Primigravid	0.84	0.59-1.19	0.318
Age (20-34 yrs)	1.28	0.53-3.06	0.584
Age (≥ 35 yrs)	1.06	0.41-2.69	0.911

Key: MP – Malaria parasite; epg- eggs per gram *significant at $\alpha_{0.05}$

4.5 IRON STATUS IN RELATION TO INFECTION

Serum samples of a subset of 189 women from the 707 recruited women were selected based on no sign of haemolysis in the sera, no history of blood transfusion in the past 8 months, no vaginal bleeding and no use of haematinics before reporting for ante-natal care. Samples were selected across the trimesters and in the groups with or without infection (Table 4.14). The iron status of these randomly selected women was determined using serum ferritin (SF), transferrin (STf) and iron (SI) levels as parameters. The mean age of this subset of women was 28.2±5.3 years with an average weight of 65.0±12.5 kg. Iron status of women in the first trimester was not included in the analysis because of the small number of available samples.

Table 4.14: Prevalence of infection in the subset of samples assayed for iron studies.

Infection status	N	First Trimester	Second Trimester	Third Trimester	Total (%)
PF	23	2 (8.7%)	17 (73.9%)	4 (17.4%)	23 (12.2%)
H	20	1 (5.0%)	13 (65.0%)	6(30.0%)	20 (10.6%)
HIV	54	1 (1.9%)	18 (33.3%)	35 (64.8%)	54 (28.6%)
PF+H	5	-	3 (60.0%)	2 (40.0%)	5 (2.6%)
PF+HIV	30	-	8 (26.7%)	22 (73.3%)	30 (15.9%)
H+HIV	3	-	1 (33.3%)	2 (66.7%)	3 (1.6%)
N	54	4 (7.4%)	29 (53.7%)	21 (38.9%)	54 (28.6%)
Total		8 (4.2%)	89 (47.1%)	92 (48.7%)	189 (100%)

4.5.1 Changes in Serum Ferritin (SF) levels in the presence of infection.

Serum Ferritin values <12ng/mL was used as cut-off among those not infected. To compensate for inflammation, the cut-off was raised to 30ng/mL for those infected (WHO, 2011). As shown in Table 4.15, the median concentration (interquartile range) of ferritin was least among the uninfected group (27.5 ng/mL (13.250-49.916)).

Median concentrations of SF was significantly higher among those with *Plasmodium* only relative to the uninfected group (p<0.0001). Similarly, SF levels in *Plasmodium* only (116.104ng/mL (34.998-197.215)) was elevated than in co-infection of *Plasmodium* and helminths (74.912ng/mL (25.992-86.596)) and also in co-infection of *Plasmodium* and HIV (50.633ng/mL (33.226-125.000)). However, the differences were not statistically significant.

A trimester-based assessment for serum ferritin among those infected in the second trimester showed the highest concentration of SF was among those infected with *Plasmodium only* (121.070ng/mL (41.614-244.814)). In comparison with the uninfected group, concentrations of SF was significantly higher among those infected with *Plasmodium* only (p<0.0001). However, co-infection of *Plasmodium* and helminths or the co-infection of *Plasmodium* and HIV had lower concentrations of SF compared with those infected with *Plasmodium* only but these were not statistically significant (Table 4.16).

In the third trimester, although the concentration of SF was highest among those infected with *Plasmodium* only (108.453ng/mL (29.727-209.000)), it was not significantly higher than the uninfected group (32.599ng/mL (14.500-40.290)). Similarly, SF concentrations were not significantly different between those with *Plasmodium* only and those with co-infection of *Plasmodium* and helminths or those with co-infection of *Plasmodium* and HIV (Table 4.16).

The median concentration of ferritin was significantly higher among those infected with helminths only (40.887ng/mL (32.316-56.753)) than the uninfected group (27.5 ng/mL (13.250-49.916). However, the SF concentrations were not significantly different between those with helminth only and those with co-infection of *Plasmodium* and helminths (74.912ng/mL (25.992-86.596)) (Table 4.15).

In the trimester-based evaluation, among those in second trimester of pregnancy, those infected with helminths only had significantly higher concentration of SF relative to

the uninfected group (p=0.017). However, the concentration of SF was not significantly different between those with helminths only and those with combined infections of *Plasmodium* and helminths (Table 4.16).

Among those in the late stage of pregnancy, there was no significant difference in the median concentrations of those with helminths only (34.534ng/mL (26.765-42.728)) and the uninfected group (32.599ng/mL (14.500-40.290)). Likewise, there was no significant difference in the concentration of SF among those with helminths only relative to those with co-infection of *Plasmodium* and helminths (Table 4.16).

Among those infected with HIV only, SF concentration was significantly higher than the uninfected group (p<0.0001). However, the concentrations of SF were not significantly different between those infected with HIV only (57.000ng/mL (30.500-117.981)) and those with co-infection of *Plasmodium* and HIV (50.633ng/mL (33.226-125.000)) (Table 4.15).

A trimester-based assessment for serum ferritin in the second trimester showed that median concentration of SF was significantly higher among those infected with HIV only relative to the uninfected group (p=0.037). However, SF concentration was lower among those infected with HIV only (52.000ng/mL (20.000-60.000)) relative to those with co-infection of *Plasmodium* and HIV (77.257ng/mL (40.878-135.891)) but the disparity was not statistically significant (Table 4.16).

In the third trimester, SF concentration was significantly higher among those infected with HIV only relative to the uninfected group (p=0.002). Similarly, SF concentration among those with HIV only (82.866ng/mL (35.000-152.000)) was higher than those with co-infection of *Plasmodium* and HIV (40.000ng/mL (22.000-125.000)) but the difference was not statistically significant (Table 4.16).

Across the different infection groups, serum ferritin values were not significantly different in mid and late stages of pregnancy; *Plasmodium* only – p=0.705, Helminth only – p=0.160, HIV only – p=0.115, *Plasmodium*/Helminths – p=0.564, *Plasmodium*/HIV – p=0.561 and uninfected – p=0.496.

Table 4.15: Median (IQ) values of serum ferritin concentrations based on infection status

Infection status	n	Median (IQ) values (ng/mL)	*P*- value
PF only	22	116.104 (34.998-197.215)[a]	p<0.0001[a]
H only	19	40.887 (32.316-56.753)[b]	0.019[b]
HIV only	28	57.000 (30.500-117.981)[c]	p<0.0001[c]
PF+H	5	74.912 (25.992-86.596)	
PF+HIV	23	50.633 (33.226-125.000)	
Uninfected	42	27.500 (13.250-49.916)[a,b,c]	

Key: IQ – Interquartile range, PF – *P. falciparum,* H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.16: Median (IQ) values of serum ferritin concentrations of those in second and third trimesters based on infection status

	Second Trimester			Third Trimester		
Infection status	n	Median (IQ) values (ng/mL)	*P*- value	n	Median (IQ) values (ng/mL)	*P*- value
PF only	16	121.070 (41.614-244.814)[a]	$p<0.0001$[a]	4	108.453 (29.727-209.000)	
H only	12	45.820 (33.838-73.039)[b]	0.017[b]	6	34.534 (26.765-42.728)	
HIV only	11	52.000 (20.000-60.000)[c]	0.037[c]	17	82.866 (35.000-152.000)[a]	0.002[a]
PF+H	3	74.912 (40.866-74.912)		2	43.155 (11.117-43.155)	
PF+HIV	8	77.257 (40.878-135.891)		15	40.000 (22.000-125.000)	
Uninfected	22	19.000 (10.000-51.934)[a,b,c]		17	32.599 (14.500-40.290)[a]	

Key: IQ – Interquartile range, PF – *P. falciparum,* H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

As shown in Table 4.17, the prevalence of iron deficiency was 22% (31/141) while there was 68.1% (96/141) prevalence of anaemia. About 26.0% (25/96) of the anaemia cases were due to iron deficiency. More so, small proportions of those who were infected with *Plasmodium* (19.2%), helminths (16%) or HIV (24.1%) were also iron deficient. Hence, the test of relationships between these infections and iron deficiency were not statistically significant. Similarly, the test of relationship between anaemia and iron deficiency was not significant.

Overall, the prevalence of iron deficiency anaemia (IDA) among the expectant mothers was 17.7% (25/141) while the cases of IDA without the compounding effect of infection was 7.1% (10/141). The prevalence of anaemia associated with infection only was 35.5% (50/141). The cases of anaemia due to unknown sources (absence of infection and no iron deficiency) was 14.9% (21/141). Iron deficiency anaemia was more prevalent among those infected with HIV only and the uninfected group (Table 4.18).

Table 4.17: Association between iron deficiency, anaemia and infections.

Ferritin levels	PF positive	PF negative	Total	χ^2	*P*-value
Iron deficient	10 (19.2%)	21 (23.6%)	31 (21.4%)		
Normal	42 (80.8%)	68 (76.4%)	110 (78.6%)		
Total	52 (100%)	89 (100%)	141 (100%)	0.365	0.546
	H positive	H negative	Total		
Iron deficient	4 (17.4%)	27 (22.9%)	31 (22%)		
Normal	19 (82.6%)	91 (77.1%)	110 (78%)		
Total	23 (100%)	118 (100%)	141 (100%)	0.338	0.561
	HIV positive	HIV negative	Total		
Iron deficient	13 (24.5%)	18 (20.5%)	31 (22%)		
Normal	40 (75.5%)	70 (79.5%)	110 (78%)		
Total	53 (100%)	88 (100%)	141 (100%)	0.320	0.572
	Anaemia present	Anaemia absent	Total		
Iron deficient	25 (26.0%)	6 (13.3%)	31 (22.0%)		
Normal	71 (74.0%)	39 (86.7%)	110 (78.0%)		
Total	96 (100%)	45 (100%)	141 (100%)	2.885	0.089

Key: PF – *P. falciparum*, H – Helminth

Table 4.18: Prevalence of anaemia and iron deficiency in relation to infection status

	n	Normal HCT/ ferritin levels	Anaemia only	Iron deficiency only	Iron deficiency anaemia
Plasmodium only	23	8 (34.8%)	12 (52.2%)	2 (8.7%)	1 (4.3%)
Helminth only	18	9 (50%)	6 (33.3%)	2 (11.1%)	1 (5.6%)
HIV only	29	4 (13.8%)	18 (62.1%)	-	7 (24.1%)
Plasmodium/ Helminth	5	1 (20%)	3 (60%)	-	1 (20%)
Plasmodium/ HIV	24	7 (29.2%)	11 (45.8%)	1 (4.2%)	5 (20.8%)
Uninfected	42	10 (23.8%)	21 (50%)	1 (2.4%)	10 (23.8%)
Total	141	39 (27.7%)	71 (50.4%)	6 (4.3%)	25 (17.7%)

4.5.2 Serum Iron (SI) levels and infections

Of the serum samples tested for iron levels, 28.8% were outside the normal range of 0.5-1.5 mg/L. The median values of those infected with *Plasmodium* only was below the normal range. Also, the single sample with *Plasmodium* and helminths co-infection also had serum iron level beneath the normal range (Table 4.19).

Those infected with *Plasmodium* only had a significantly lower serum iron level relative to the uninfected (p=0.025). Similarly, the median serum iron levels of those infected with *Plasmodium* only (0.3mg/L) was significantly lower than those with co-infections of *Plasmodium* and HIV (1.095 (0.840-1.350) (Table 4.19).

In a trimester-based assessment, in the second trimester, the group with *Plasmodium* infection only had SI level less than the uninfected group but the difference was not significant. Likewise, the group with *Plasmodium* infection only (0.33mg/L) had SI levels lesser than the group with co-infection of *Plasmodium* and HIV (1.470mg/L (1.360-1.470)) and SI levels higher than the group with *Plasmodium* and helminths (0.18mg/L) but the differences were not statistically significant (Table 4.20). In the third trimester, the least concentration of SI remained in the group with *Plasmodium* infection only though it was not significantly reduced relative to the uninfected group (1.220mg/L (0.330-1.270)). However, SI levels in the group with *Plasmodium* infection only was significantly lower than those with co-infection of *Plasmodium* and HIV (p=0.026) (Table 4.20).

Among those infected with helminths only, the level of SI was greater than the uninfected group but the difference was not significant (Table 4.19). The median SI level in those infected with helminth only could not be compared with those with co-infection of *Plasmodium* and helminth due to its small sample size (Table 4.19).

In the second trimester, those infected with helminth only (1.36mg/L (1.200-1.360)) had SI concentration higher than the uninfected group (1.240mg/L (0.940-1.340)) and the group with co-infection of *Plasmodium* and helminths (0.180mg/L) (Table 4.20). However, these differences were not statistically significant.

HIV infection only (1.005mg/L (0.630-1.265)) showed a lower SI level relative to the uninfected group (1.240mg/L (0.940-1.340)) but the difference was not statistically

significant. Similarly, the difference was not statistically significant among those with HIV only and those with co-infection of *Plasmodium* and HIV (Table 4.19).

In the trimester-based assessment, the SI level was slightly lower in those infected with HIV only (1.200mg/L (0.990-1.640)) in the second trimester relative to the uninfected (1.280mg/L (1.240-1.540)) and the group with *Plasmodium* and HIV co-infection (1.470mg/L (1.360-1.470)) but the differences were not significant (Table 4.20). Likewise in the third trimester, those infected with HIV only (0.720mg/L (0.570-1.110)) had SI levels lower than the uninfected (1.220mg/L (0.330-1.270)) and those with co-infection of *Plasmodium* and HIV (0.900mg/L (0.820-1.280)). Also, these differences were not statistically significant (Table 4.20).

Comparative analysis of the mid and late gestational stages showed there were significantly higher levels of serum iron in second trimester relative to the third trimester among those with HIV only – p=0.010, *Plasmodium*/HIV – p=0.018 and the uninfected group – p=0.032.

Table 4.19: Median (IQ) values of serum iron concentrations based on infection status

Infection status	n	Median (IQ) values (mg/L)	*P*- value
PF only	3	0.300 (0.300-0.300)[a,b]	0.025[a]
H only	3	1.360 (1.200-1.360)	
HIV only	26	1.005 (0.630-1.265)	
PF+H	1	0.180 (0.180-0.180)	
PF+HIV	16	1.095 (0.840-1.350)[b]	0.008[b]
Uninfected	12	1.240 (0.940-1.340)[a]	

Key: IQ – Interquartile range, PF – *P. falciparum,* H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.20: Median (IQ) values of serum iron concentrations of those in second and third trimesters based on infection status

	Second Trimester			Third Trimester		
Infection status	n	Median (IQ) values (ng/mL)	*P*- value	n	Median (IQ) values (ng/mL)	*P*- value
PF only	1	0.330 (0.330-0.330)		2	0.300 (0.300-0.300)[a]	0.026[a]
H only	3	1.36 (1.200-1.360)		-		
HIV only	11	1.200 (0.990-1.640)		15	0.720 (0.570-1.110)	
PF+H	1	0.180 (0.180-0.180)		-		
PF+HIV	3	1.470 (1.360-1.470)		13	0.900 (0.820-1.280)[a]	
Uninfected	16	1.280 (1.240-1.540)		17	1.220 (0.330-1.270)	

Key: IQ – Interquartile range, PF – *P. falciparum,* H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.21 shows the relationship between serum iron levels, iron deficiency, anaemia and infections. Among those with *Plasmodium* infection, 30% had serum iron levels that was not within the normal range (0.5-1.5 mg/L). However, the relationship between *Plasmodium* infection and iron levels was not statistically significant (p=0.887). Furthermore, an equal proportion of those with helminths had iron levels outside the normal range. Similarly, a low proportion of those with HIV (20.5%) had serum iron levels that was not within the normal range but the association was not significant (p=0.070).

There was also no significant association between iron deficiency and serum iron levels as the number of those with iron deficiency and abnormal iron levels was low (14.3%). However, a very strong association existed between anaemia and iron levels (Table 4.21).

Table 4.21: Association between abnormal iron levels, iron deficiency, anaemia and infections.

	PF positive	PF negative	Total	χ^2	*P*-value
Abnormal level	6 (30.0%)	17 (28.3%)	23 (28.8%)		
Normal	14 (70.0%)	43 (71.7%)	57 (71.3%)		
Total	20 (100%)	60 (100%)	80 (100%)	0.020	0.887
	H positive	H negative	Total		
Abnormal level	2 (50.0%)	21 (27.6%)	23 (28.8%)		
Normal	2 (50.0%)	55 (72.4%)	57 (71.3%)		
Total	4 (100%)	76 (100%)	80 (100%)	0.928	0.335
	HIV positive	HIV negative	Total		
Abnormal level	9 (20.5%)	14 (38.9%)	23 (28.8%)		
Normal	35 (79.5%)	22 (61.1%)	57 (71.3%)		
Total	44 (100%)	36 (100%)	80 (100%)	3.285	0.070
	Iron deficient	Normal	Total		
Abnormal level	3 (14.3%)	20 (35.1%)	23 (29.5%)		
Normal	18 (85.7%)	37 (64.9%)	55 (70.5%)		
Total	21 (100%)	57 (100%)	78 (100%)	3.194	0.074
	Anaemia present	Anaemia absent	Total		
Abnormal level	11 (18.6%)	10 (52.6%)	21 (26.9%)		
Normal	48 (81.4%)	9 (47.4%)	57 (73.1%)		
Total	59 (100%)	19 (100%)	78 (100%)	8.438	0.004*

Key: PF – *P. falciparum*, H – Helminth *significant at $\alpha_{0.05}$

4.5.3 Serum Transferrin (STf) levels in relation to infection status

The median concentration (interquartile range) of serum transferrin (STf) was lesser among those infected with *Plasmodium* only (5843.680µg/mL (4545.710-7114.688)) relative to the uninfected group (6572.960µg/mL (5425.080-7296.525)) and the group with co-infection of *Plasmodium* and helminths (6772.475µg/mL (6246.165-8479.938)) but the differences were not statistically significant. However, median STf concentration was significantly higher among those infected with *Plasmodium* only than those with co-infection of *Plasmodium* and HIV ($p<0.0001$) (Table 4.22).

In the trimester-based assessment for those in the second trimester, the median STf concentration was reduced among those infected with *Plasmodium* only (5782.065µg/mL (4545.710-6942.720)) relative to the uninfected group (6753.880µg/mL (5743.750-7296.525)) and the group with co-infection of *Plasmodium* and helminths (6574.665µg/mL (6147.260-6574.665)). However, these differences were not statistically significant. Furthermore, those infected with *Plasmodium* only had STf concentration that was significantly higher than those with co-infection of *Plasmodium* and HIV ($p=0.025$) (Table 4.23).

In third trimester, the median STf values was highest among those infected with *Plasmodium* only (8441.255µg/mL (6865.360-8441.255)) but it was not significantly different from the uninfected group (6542.880µg/mL (4014.510-7771.010)) and the group with co-infection of *Plasmodium* and helminths (7757.720µg/mL (6542.880-7757.720)). However, those infected with *Plasmodium* only had STf concentration that was significantly higher than those with co-infection of *Plasmodium* and HIV ($p=0.030$) (Table 4.23).

STf levels was lower among those infected with helminths only (5849.085µg/mL (4527.050-6793.285)) relative to the uninfected group (6572.960µg/mL (5425.080-7296.525)) and the group with co-infection of *Plasmodium* and helminths (6772.475µg/mL (6246.165-8479.938)) but the differences were not statistically significant. However, median STf concentration was significantly higher among those infected with helminths only than those with co-infection of helminths and HIV ($p=0.028$) (Table 4.22).

In the trimester-based assessment for those in the second trimester, STf concentration was lower in those infected with helminths only (6050.420µg/mL (4106.480-

6761.030)) relative to the STf concentration of the uninfected group (6753.880µg/mL (5743.750-7296.525)) and the group with co-infection of *Plasmodium* and helminths (6574.665µg/mL (6147.260-6574.665)) but the differences were not statistically significant. However, those infected with helminths only had STf concentration that was higher than those with co-infection of helminths and HIV but the difference was also not statistically significant (Table 4.23). Among those in the third trimester, STf levels was lower among those infected with helminths only (5985.950µg/mL (5289.000-7510.545)) relative to the uninfected group (6542.880µg/mL (4014.510-7771.010)) and the group with co-infection of *Plasmodium* and helminths (7757.720µg/mL (6542.880-7757.720)) but the differences were not significant. However, those infected with helminths only had STf concentration that was higher than those with co-infection of helminths and HIV but the difference was also not statistically significant (Table 4.23).

Serum transferrin (STf) levels was significantly lower among those infected with HIV only (2554.590µg/mL (2119.935-3040.485)) relative to the uninfected group (6572.960µg/mL (5425.080-7296.525)). However, STf levels in those infected with HIV only was not significantly different from those with co-infection of helminths and HIV (2964.390µg/mL (2205.900-2964.390)) and those with co-infection of *Plasmodium* and HIV (2756.950µg/mL (2311.905-3289.235)) (Table 4.22).

In the trimester-based assessment for those in the second trimester, those infected with HIV only had significantly lower STf concentration (2689.835µg/mL (2159.943-3066.178) relative to the uninfected group (6753.880µg/mL (5743.750-7296.525)). However, STf concentration was higher in those infected with HIV only relative to those with co-infection of *Plasmodium* and HIV (2177.330µg/mL (2096.150-2177.330)) but the difference was not significant (Table 4.23). Among those in the third trimester, those infected with HIV only had STf levels significantly lower than the uninfected group ($p=0.003$). STf levels was lower in those infected with HIV only (2528.720µg/mL (2163.780-2979.090)) relative to those with co-infection of helminths and HIV (3190.800µg/mL (2964.390-3190.800)) and those with co-infection of *Plasmodium* and HIV (2865.750µg/mL (2627.190-3503.540)) but the differences were not statistically significant (Table 4.23).

Across the different infection groups, serum transferrin values were not significantly altered by changes in trimesters; *Plasmodium* only – p=0.122, Helminth only – p=0.430, HIV only – p=0.791, *Plasmodium*/Helminths – p=0.439, Helminths/HIV – p=0.221, *Plasmodium*/HIV – p=0.076 and uninfected – p=0.867).

Table 4.22: Median (IQ) values of serum transferrin concentrations based on infection status

Infection status	n	Median (IQ) values (µg/mL)	*P*- value
PF only	20	5843.680 (4545.710-7114.688)[c]	p<0.0001[c]
H only	20	5849.085 (4527.050-6793.285)[b]	0.028[b]
HIV only	28	2554.590 (2119.935-3040.485)[a]	p<0.0001[a]
PF+H	4	6772.475 (6246.165-8479.938)	
H+HIV	3	2964.390 (2205.900-2964.390)[b]	
PF+HIV	13	2756.950 (2311.905-3289.235)[c]	
Uninfected	25	6572.960 (5425.080-7296.525)[a]	

Key: IQ – Interquartile range, PF – *P. falciparum,* H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.23: Median (IQ) values of serum transferrin concentrations of those in second and third trimesters based on infection status

	Second Trimester			Third Trimester		
Infection status	n	Median (IQ) values (ng/mL)	*P*- value	n	Median (IQ) values (ng/mL)	*P*- value
PF only	16	5782.065 (4545.710-6942.720)[b]	0.025[b]	2	8441.255 (6865.360-8441.255)[b]	0.030[b]
H only	13	6050.420 (4106.480-6761.030)		6	5985.950 (5289.000-7510.545)	
HIV only	8	2689.835 (2159.943-3066.178)[a]	0.005[a]	19	2528.720 (2163.780-2979.090)[a]	0.003[a]
PF+H	2	6574.665 (6147.260-6574.665)		2	7757.720 (6542.880-7757.720)	
H+HIV	1	2205.900 (2205.900-2205.900)		2	3190.800 (2964.390-3190.800)	
PF+HIV	2	2177.330 (2096.150-2177.330)[b]		11	2865.750 (2627.190-3503.540)[b]	
Uninfected	13	6753.880 (5743.750-7296.525)[a]		9	6542.880 (4014.510-7771.010)[a]	

Key: IQ – Interquartile range, PF – *P. falciparum,* H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

4.6 CYTOKINE PROFILE MODIFICATION IN RESPONSE TO SINGLE AND CO-INFECTIONS

Serum samples of a subset of one hundred and thirty parturient women across the groups were used for cytokine profile analysis. Twenty-two (16.9%) of the selected samples had *Plasmodium* only, 21 (16.2%) was helminths only, 29 (22.3%) had HIV only and 29 (22.3%) were uninfected. The remaining were co-infections of *Plasmodium*/helminths (3.8%), *Plasmodium*/HIV (15.4%) and helminth/HIV (3.1%). Nine (6.9%) were in early gestation, 60 (46.2%) in the mid gestation and 61 (46.9%) in the late gestational stage (Table 4.24). Cytokine profile was evaluated using an array of cytokines consisting of pro-inflammatory cytokines (PICs): TNF-α, IFN-γ, IL-1α, IL-2, IL-6, IL-12(p70) and IL-17 and anti-inflammatory cytokines (AICs): IL-4, IL-10 and IL-13. Due to the small number of samples in early stage of pregnancy, they were not included in the cytokine data analysis.

Table 4.24: Prevalence of infection by gestational age.

	First Trimester	Second Trimester	Third Trimester	Total (%)
PF	2 (22.2%)	13 (21.7%)	7 (11.5%)	22 (16.9%)
H	1 (11.1%)	13 (21.7%)	7 (11.5%)	21 (16.2%)
HIV	1 (11.1%)	8 (13.3%)	20 (32.8%)	29 (22.3%)
PF+H	-	3 (5.0%)	2 (3.3%)	5 (3.8%)
PF+HIV	-	8 (13.3%)	12 (19.7%)	20 (15.4%)
H+HIV	-	1 (1.7%)	3 (4.9%)	4 (3.1%)
N	5 (55.6%)	14 (23.3%)	10 (16.4%)	29 (22.3%)
Total	9 (6.9%)	60 (46.2%)	61 (46.9%)	130 (100%)

Key: PF= *Plasmodium,* H= Helminths, HIV – Human Immunodeficiency Virus

4.6.1 Pro-inflammatory cytokine (PIC) profile modification in response to *Plasmodium* infection and its co-infection with helminths or HIV

The median concentrations of all the PICs were not significantly different between those with *Plasmodium* only and the uninfected. However, in comparison to the group with co-infection of *Plasmodium* and helminths, the concentrations of IFN-γ was significantly higher and IL-2 was significantly lower among those with *Plasmodium* only. The differences in concentrations of TNF-α**,** IL-1α, IL-6, IL-12(p70) and IL-17 were not statistically significant between the two groups (Table 4.25). Relative to those with co-infection of *Plasmodium* and HIV, concentrations of IL-12(p70) was significantly higher while IL-1α and IL-17 were significantly lower in those with *Plasmodium* only. The differences in levels of TNF-α**,** IFN-γ, IL-2 and IL-6 were not statistically significant between these groups (Table 4.25).

In the second trimester, median concentrations of all the PICs were not significantly different among those with *Plasmodium* only relative to the uninfected. In comparison to the group with co-infection of *Plasmodium* and helminths, only the concentration of IFN-γ was considerably greater among those infected with *Plasmodium* only. The concentrations of TNF-α, IL-1α, IL-2, IL-6, IL-12(p70) and IL-17 in the two groups were not significantly different (Table 4.26). In the group with co-infection of *Plasmodium* and HIV, concentrations of IL-17 was significantly higher and IL-12(p70) was significantly lower relative to those with *Plasmodium* only. The concentrations of TNF-α**,** IFN-γ, IL-1α, IL-2 and IL-6 were not significantly different (Table 4.26).

Similar to the second trimester, changes in the median concentrations of all the PICs measured for those in third trimester were not significantly different between the group with *Plasmodium* only and the uninfected (Table 4.26). In comparison to the group with co-infection of *Plasmodium* and helminths, only the concentration of IL-2 was significantly lower among those with *Plasmodium* infection only. The concentrations of TNF-α, IFN-γ, IL-1α, IL-6, IL-12(p70) and IL-17 were not significantly different between the two groups (Table 4.26). Among those infected with *Plasmodium* only, the level of IL-12(p70) was significantly higher while the concentration of IL-17 was significantly lower than those with co-infection of *Plasmodium* and HIV. The levels of TNF-α, IFN-γ, IL-1α, IL-2 and IL-6 were not significantly different between these groups (Table 4.26).

Significant variations in these cytokines at mid and late stages of pregnancy were in the uninfected groups (IL-17; $p = 0.045$) and the group with co-infection of *Plasmodium* and HIV (TNF-α; p=0.012, IL-6; p=0.017).

Table 4.25: Median (IQ) values of PIC concentrations of those with *Plasmodium* and its co-infection with helminths or HIV

	PF only (n=22)	PF+H (n=5)	PF+HIV (n=20)	Uninfected (n=29)	*P*- value
TNF-α (ng/mL)	0.023 (0.005-0.088)	0.047 (0.039-0.062)	0.037 (0.027-0.075)	0.007 (0.001-0.043)	
IFN-γ (ng/mL)	0.002 (0.002-0.003)[a]	0.0004 (0.0004-0.017)[a]	0.003 (0.0002-0.008)	0.002 (0.002-0.005)	0.024[a]
IL-1α (pg/mL)	0.115 (0.033-0.176)[c]	0.080 (0.068-0.144)	0.286 (0.097-0.324)[c]	0.103 (0.045-0.232)	0.014[c]
IL-2 (pg/mL)	178.135 (47.309-446.694)[b]	709.642 (709.642-1594.226)[b]	192.529 (12.917-377.415)	55.514 (55.514-55.514)	0.005[b]
IL-6 (ng/mL)	0.017 (0.016-0.019)	0.020 (0.016-0.023)	0.017 (0.016-0.018)	0.017 (0.016-0.019)	
IL-12(p70) (pg/mL)	33.800 (15.044-89.313)[e]	56.607 (20.483-493.558)	<2.000[e]	23.478 (15.980-57.790)	p<0.0001[e]
IL-17 (pg/mL)	22.216 (15.132-33.785)[d]	32.0370 (19.113-62.619)	47.3060 (37.570-53.814)[d]	18.6700 (11.600-32.892)	p<0.0001[d]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.26: Median (IQ) values of PIC concentrations of those in second and third trimesters with *Plasmodium* and its co-infection with helminths or HIV

Second trimester	**PF only (n=13)**	**PF+H (n=3)**	**PF+HIV (n=8)**	**Uninfected (n=14)**	***P*- value**
TNF-α (ng/mL)	0.045 (0.005-0.084)	0.047 (0.031-0.047)	0.027 (0.027-0.027)	0.0102 (0.001-0.037)	
IFN-γ (ng/mL)	0.002 (0.002-0.003)[a]	0.0004 (0.0004-0.00040)[a]	0.004 (0.0002-0.013)	0.002 (0.002-0.004)	0.005[a]
IL-1α (pg/mL)	0.115 (0.039-0.191)	0.068 (0.068-0.068)	0.286 (0.097-0.315)	0.115 (0.074-0.247)	
IL-2 (pg/mL)	218.503 (47.309-444.237)	709.642 (709.642-709.642)	541.570 (171.426-541.570)	55.514 (55.514-266.989)	
IL-6 (ng/mL)	0.018 (0.016-0.019)	0.018 (0.014-0.018)	0.016 (0.015-0.017)	0.016 (0.016-0.019)	
IL-12(p70) (pg/mL)	28.273 (14.644-135.196)[c]	56.607 (33.800-56.607)	<2.000[c]	23.868 (19.139-55.112)	0.023[c]
IL-17 (pg/mL)	23.992 (16.460-36.019)[b]	19.556 (18.670-19.556)	57.074 (53.814-57.074)[b]	15.132 (11.600-31.108)	0.029[b]
Third trimester	**PF only (n=7)**	**PF+H (n=2)**	**PF+HIV (n=12)**	**Uninfected (n=10)**	***P*- value**
TNF-α (ng/mL)	0.007 (0.005-0.096)	0.054 (0.047-0.054)	0.055 (0.035-0.082)	0.001(0.001-0.077)	
IFN-γ (ng/mL)	0.002 (0.002-0.002)	0.016 (0.0004-0.016)	0.003 (0.0002-0.006)	0.002 (0.002-0.004)	
IL-1α (pg/mL)	0.138 (0.022-0.351)	0.144 (0.091-0.144)	0.292 (0.103-0.354)	0.085 (0.048-0.156)	
IL-2 (pg/mL)	47.309 (47.309-47.309)[a]	1087.664 (709.642-1087.664)[a]	145.974 (12.917-333.426)	55.514 (55.514-55.514)	0.046[a]
IL-6 (ng/mL)	0.017 (0.016-0.018)	0.021 (0.020-0.021)	0.018 (0.016-0.019)	0.017 (0.015-0.017)	
IL-12 (p70) (pg/mL)	32.498 (24.258-32.498)[c]	323.825 (16.044-323.825)	<2.000[c]	17.458 (14.644-54.922)	p<0.0001[c]
IL-17 (pg/mL)	23.992 (16.460-36.019)[b]	56.290 (43.632-56.290)	44.058 (32.718-53.814)[b]	30.219 (17.786-40.238)	0.026[b]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus; Similar superscripts show significant difference at $\alpha_{0.05}$

4.6.2 Pro-inflammatory cytokine (PIC) profile modification in response to Helminth infections and its co-infection with *Plasmodium* or HIV

The levels of TNF-α and IL-2 were significantly higher and IFN-γ was significantly lower among those infected with helminths only relative to the uninfected. The differences in concentrations of IL-1α, IL-6, IL-12(p70) and IL-17 were not statistically significant (Table 4.27). However, in comparison to the group with co-infection of *Plasmodium* and helminths, only the concentration of IL-2 was significantly lower among those infected with helminths only. The differences in levels of the other PICs: TNF-α**,** IFN-γ, IL-1α, IL-6, IL-12(p70) and IL-17 were not statistically significant (Table 4.27). Relative to those with co-infection of helminths and HIV, levels of IL-1α and IL-12(p70) were significantly higher while TNF-α, IFN-γ and IL-17 were significantly lower in those with helminths only. The differences in levels of IL-2 and IL-6 were not statistically significant between these groups (Table 4.27).

In the second trimester, median concentrations of TNF-α, IL-2 and IL-17 were significantly higher while IFN-γ was significantly lower among those infected with helminths only relative to the uninfected. The concentrations of IL-1α, IL-6 and IL-12(p70) were not significantly different in the two groups (Table 4.28). In comparison to the group with co-infection of *Plasmodium* and helminths, only the concentration of IFN-γ was significantly higher among those infected with helminths only. The concentrations of TNF-α, IL-1α, IL-2, IL-6, IL-12(p70) and IL-17 were not significantly different between them (Table 4.28). For the group with co-infection of helminths and HIV, significant differences in concentrations of cytokines relative to the helminth only group could not be determined as there was a single sample of helminths and HIV co-infection (Table 4.28).

Median concentration of IL-2 in the third trimester was significantly higher among those with helminths only relative to the uninfected. Alterations in the levels of TNF-α, IFN-γ, IL-1α, IL-6, IL-12(p70) and IL-17 were not significantly different between them (Table 4.28). In comparison to the group with co-infection of *Plasmodium* and helminths, only the concentration of IL-6 was significantly lower among those with helminth infection only. The concentrations of TNF-α, IFN-γ, IL-1α, IL-2, IL-12(p70) and IL-17 were not significantly different in the two groups (Table 4.28). Relative to those with co-infection of helminths and HIV, concentrations of IL-1α and IL-12(p70)

were significantly lower while IFN-γ and IL-17 were significantly lower in the group with helminths only. The concentrations of TNF-α, IL-2 and IL-6 were not significantly different between these groups (Table 4.28).

Significant difference in mid and late pregnancy profiles of these cytokines was in Helminth only group (IFN-γ; $p = 0.043$).

Table 4.27: Median (IQ) values of PIC concentrations of those with Helminth and its co-infection with *Plasmodium* or HIV

	H only (n=21)	PF+H (n=5)	H+HIV (n=4)	Uninfected (n=29)	*P*- value
TNF-α (ng/mL)	0.049 (0.019-0.107)[a,e]	0.047 (0.039-0.062)	0.186 (0.169-0.224)[e]	0.007 (0.001-0.043)[a]	0.004[a] 0.027[e]
IFN-γ (ng/mL)	0.001 (0.001-0.003)[b,g]	0.0004 (0.0004-0.017)	0.007 (0.007-0.032)[g]	0.002 (0.002-0.005)[b]	0.001[b] 0.004[g]
IL-1α (pg/mL)	0.091 (0.068-0.197)[f]	0.080 (0.068-0.144)	0.006 (0.006-0.006)[f]	0.103 (0.045-0.232)	0.002[f]
IL-2 (pg/mL)	229.639 (74.059-661.799)[c,d]	709.642 (709.642-1594.226)[d]	1849.023 (177.959-1849.023)	55.514 (55.514-55.514)[c]	p<0.0001[c] 0.022[d]
IL-6 (ng/mL)	0.017 (0.016-0.018)	0.020 (0.016-0.023)	0.018 (0.017-0.025)	0.017 (0.016-0.019)	
IL-12(p70) (pg/mL)	23.478 (15.744-53.706)[j]	56.607 (20.483-493.558)	<2.000[j]	23.478 (15.980-57.789)	0.006[j]
IL-17 (pg/mL)	31.108 (21.329-34.678)[h]	32.037 (19.113-62.619)	60.395 (37.570-60.395)[h]	18.670 (11.600-32.892)	0.011[h]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.28: Median (IQ) values of PIC concentrations of those in second and third trimesters with Helminth and its co-infection with *Plasmodium* or HIV

Second trimester	H only (n=13)	PF+H (n=3)	H+HIV (n=1)	Uninfected (n=14)	*P*- value
TNF-α (ng/mL)	0.053 (0.047-0.124)[c]	0.047 (0.031-0.047)	0.184 (0.184-0.184)	0.0102 (0.001-0.037)[c]	0.015[c]
IFN-γ (ng/mL)	0.001 (0.001-0.001)[d,e]	0.0004 (0.0004-0.00040)[e]	0.007 (0.007-0.007)	0.002 (0.002-0.004)[d]	p<0.0001[d] 0.035[e]
IL-1α (pg/mL)	0.091 (0.068-0.126)	0.068 (0.068-0.068)	0.006 (0.006-0.006)	0.115 (0.074-0.247)	
IL-2 (pg/mL)	229.639 (74.059-793.276)[a]	709.642 (709.642-709.642)	1849.023 (1849.023-1849.023)	55.514 (55.514-266.989)[a]	0.023[a]
IL-6 (ng/mL)	0.017 (0.015-0.018)	0.018 (0.014-0.018)	0.017 (0.017-0.017)	0.016 (0.016-0.019)	
IL-12(p70) (pg/mL)	24.258 (16.077-45.372)	56.607 (33.800-56.607)	<2.000	23.868 (19.139-55.112)	
IL-17 (pg/mL)	29.326 (20.443-35.572)[b]	19.556 (18.670-19.556)	37.570 (37.570-37.570)	15.132 (11.600-31.108)[b]	0.038[b]
Third trimester	**H only (n=7)**	**PF+H (n=2)**	**H+HIV (n=3)**	**Uninfected (n=10)**	***P*- value**
TNF-α (ng/mL)	0.029 (0.019-0.081)	0.054 (0.047-0.054)	0.189 (0.164-0.189)	0.001 (0.001-0.077)	
IFN-γ (ng/mL)	0.002 (0.001-0.004)[b]	0.016 (0.0004-0.016)	0.007 (0.007-0.007)[b]	0.002 (0.002-0.004)	0.038[b]
IL-1α (pg/mL)	0.091 (0.068-0.304)[c]	0.144 (0.091-0.144)	0.006 (0.006-0.006)[c]	0.085 (0.048-0.156)	0.011[c]
IL-2 (pg/mL)	395.070 (74.059-900.758)[e]	1087.664 (709.642-1087.664)	1485.912 (177.959-1485.912)	55.514 (55.514-55.514)[e]	0.009[e]
IL-6 (ng/mL)	0.016 (0.016-0.019)[a]	0.021 (0.020-0.021)[a]	0.018 (0.018-0.018)	0.017 (0.015-0.017)	0.049[a]
IL-12(p70) (pg/mL)	18.722 (15.164-81.490)[f]	323.825 (16.044-323.825)	<2.000[f]	17.458 (14.644-54.922)	0.039[f]
IL-17 (pg/mL)	32.892 (20.442-34.678)[d]	56.290 (43.632-56.290)	63.633 (60.395-63.633)[d]	30.219 (17.786-40.238)	0.040[d]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus; Similar superscripts show significant difference at $\alpha_{0.05}$

4.6.3 Pro-inflammatory cytokine (PIC) profile modification in response to HIV infection and its co-infection with *Plasmodium* or Helminths

The median levels of TNF-α, IL-6 and IL-17 were significantly higher while IL-1α and IL-12(p70) were significantly lower among those infected with HIV only relative to the uninfected. The difference in concentrations of IFN-γ and IL-2 were not statistically significant (Table 4.29). In comparison to the group with co-infection of *Plasmodium* and HIV, levels of TNF-α**,** IL-6, IL-12(p70) and IL-17 were significantly higher while IL-1α was significantly lower among those infected with HIV only. The differences in concentrations of the other PICs: IFN-γ and IL-2 were not statistically significant between the two groups (Table 4.29). Relative to those with co-infection of helminths and HIV, concentration of IL-12(p70) was significantly higher while IFN-γ was significantly lower in those with HIV only. The concentrations of TNF-α**,** IL-1α, IL-2, IL-6 and IL-17 were not significantly different between these groups (Table 4.29).

In the second trimester, median levels of TNF-α, IL-6 and IL-17 were significantly higher while IL-1α and IL-12(p70) were significantly lower among those infected with HIV only relative to the uninfected. The concentrations of IFN-γ and IL-2 were not significantly different between these groups (Table 4.30). In comparison to the group with co-infection of *Plasmodium* and HIV, levels of TNF-α, IL-6 and IL-12(p70) were significantly higher while IL-1α was significantly lower among those infected with HIV only. The concentrations of IFN-γ, IL-2 and IL-17 were not significantly different between these groups (Table 4.30). For the group with co-infection of helminths and HIV, significant differences in concentrations of cytokines relative to the HIV only group could not be determined as there was a single sample of helminths and HIV co-infection (Table 4.30).

In the late stage of parturition, median levels of TNF-α, IL-6 and IL-17 were significantly higher while IL-1α was significantly lower among those infected with HIV only relative to the uninfected. The concentrations of IFN-γ, IL-12(p70) and IL-17 were not significantly different between these groups (Table 4.30). In comparison to the group with co-infection of *Plasmodium* and HIV, concentrations of TNF-α, IL-12(p70) and IL-17 were significantly higher while IL-1α was significantly lower among those infected with HIV only. The concentrations of IFN-γ, IL-2 and IL-6 were not significantly different between these groups (Table 4.30). Relative to those with

combined infections of helminths and HIV, only the concentration of IL-12(p70) was significantly higher among those with HIV only. The concentrations of TNF-α, IFN-γ, IL-1α, IL-2, IL-6 and IL-17 were not significantly different between these groups (Table 4.30).

Differences in trimesters showed significant variations in concentrations of TNF-α (p=0.012) and IL-6 (p=0.017) in *Plasmodium* and HIV co-infection group.

Table 4.29: Median (IQ) values of PIC concentrations of those with HIV and its co-infection with *Plasmodium* or Helminth

	HIV only (n=29)	PF+HIV (n=20)	H+HIV (n=4)	Uninfected (n=29)	*P*- value
TNF-α (ng/mL)	0.189 (0.173-0.219)[a,f]	0.037 (0.027-0.075)[a]	0.186 (0.169-0.224)	0.007 (0.001-0.043)[f]	p<0.0001[a,f]
IFN-γ (ng/mL)	0.0004 (0.0004-0.006)[e]	0.003 (0.0002-0.008)	0.007 (0.007-0.032)[e]	0.002 (0.002-0.005)	0.020[e]
IL-1α (pg/mL)	0.006 (0.006-0.008)[c,h]	0.286 (0.097-0.324)[c]	0.006 (0.006-0.006)	0.103 (0.045-0.232)[h]	p<0.0001[c,h]
IL-2 (pg/mL)	183.703 (8.360-534.534)	192.529 (12.917-377.415)	1849.023 (177.959-1849.023)	55.514 (55.514-55.514)	
IL-6 (ng/mL)	0.019 (0.018-0.021)[b,g]	0.017 (0.016-0.018)[b]	0.018 (0.017-0.025)	0.017 (0.016-0.019)[g]	0.003[b] 0.001[g]
IL-12(p70) (pg/mL)	16.044 (16.044-16.044)[k]	<2.000[l]	<2.000[m]	23.478 (15.980-57.789)[k]	0.018[k] p<0.0001[l,m]
IL-17 (pg/mL)	60.334 (47.306-66.870)[d,j]	47.306 (37.570-53.814)[d]	60.395 (37.570-60.395)	18.670 (11.600-32.892)[j]	0.005[d] p<0.0001[j]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.30: Median (IQ) values of PIC concentrations of those in second and third trimesters with HIV and its co-infection with *Plasmodium* or Helminths

Second trimester	**HIV only (n=8)**	**PF + HIV (n=8)**	**H+HIV (n=1)**	**Uninfected (n=14)**	***P*- value**
TNF-α (ng/mL)	0.201 (0.183-0.231)^{a,d}	0.027 (0.027-0.027)^{a}	0.184 (0.184-0.184)	0.0102 (0.001-0.037)^{d}	p<0.0001^{a,d}
IFN-γ (ng/mL)	0.001 (0.0004-0.007)	0.004 (0.0002-0.013)	0.007 (0.007-0.007)	0.002 (0.002-0.004)	
IL-1α (pg/mL)	0.006 (0.006-0.041)^{c,e}	0.286 (0.097-0.315)^{c}	0.006 (0.006-0.006)	0.115 (0.074-0.247)^{e}	0.003^{c}; 0.002^{e}
IL-2 (pg/mL)	125.808 (8.36-313.280)	541.570 (171.426-541.570)	1849.023 (1849.023-1849.023)	55.514 (55.514-266.989)	
IL-6 (ng/mL)	0.019 (0.018-0.037)^{b,f}	0.016 (0.015-0.017)^{b}	0.017 (0.017-0.017)	0.016 (0.016-0.019)^{f}	0.001^{b}; 0.003^{f}
IL-12(p70) (pg/mL)	16.044 (16.044-16.044)^{h,j}	<2.000^{j}	<2.000	23.868 (19.139-55.112)^{h}	0.018^{h}; 0.010^{j}
IL-17 (pg/mL)	61.733 (46.497-68.508)^{g}	57.074 (53.814-57.074)	37.570 (37.570-37.570)	15.132 (11.600-31.108)^{g}	p<0.0001^{g}
Third trimester	**HIV only (n=20)**	**PF + HIV (n=12)**	**H+HIV (n=3)**	**Uninfected (n=10)**	***P*- value**
TNF-α (ng/mL)	0.177 (0.159-0.239)^{a,d}	0.055 (0.035-0.082)^{a}	0.189 (0.164-0.189)	0.001 (0.001-0.077)^{d}	0.001^{a}; 0.006^{d}
IFN-γ (ng/mL)	0.0004 (0.0004-0.010)	0.003 (0.0002-0.006)	0.007 (0.007-0.007)	0.002 (0.002-0.004)	
IL-1α (pg/mL)	0.006 (0.006-0.006)^{b,e}	0.292 (0.103-0.354)^{b}	0.006 (0.006-0.006)	0.085 (0.048-0.156)^{e}	p<0.0001^{b,e}
IL-2 (pg/mL)	196.998 (8.36-721.031)	145.974 (12.917-333.426)	1485.912 (177.959-1485.912)	55.514 (55.514-55.514)	
IL-6 (ng/mL)	0.018 (0.017-0.020)^{f}	0.018 (0.016-0.019)	0.018 (0.018-0.018)	0.017 (0.015-0.017)^{f}	0.026^{f}
IL-12(p70) (pg/mL)	16.044 (16.044-16.044)^{h,j}	<2.000^{j}	<2.000^{h}	17.458 (14.644-54.922)	0.004^{h}; p<0.0001^{j}
IL-17 (pg/mL)	60.334 (47.306-66.870)^{c,g}	44.058 (32.718-53.814)^{c}	63.633 (60.395-63.633)	30.219 (17.786-40.238)^{g}	0.004^{c}; p<0.0001^{g}

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus; Similar superscripts show significant difference at $\alpha_{0.05}$

4.6.4 Anti-inflammatory cytokine (AIC) profile modification in response to *Plasmodium* and its co-infection with helminths or HIV

The level of IL-13 was significantly higher among those infected with *Plasmodium* only relative to the uninfected while IL-4 and IL-10 were not significantly different between these groups. However, comparison of those with *Plasmodium* only and those with co-infection of *Plasmodium* and helminths, showed that the concentrations of all the AIC were not significantly different between these groups (Table 4.31). Relative to those with co-infection of *Plasmodium* and HIV, concentration of IL-10 was significantly lower in those with *Plasmodium* only. The differences in concentrations of IL-4 and IL-13 were not statistically significant between these groups (Table 4.31)

In the second trimester, median level of IL-13 was significantly higher among those with *Plasmodium* only than in the uninfected while IL-4 and IL-10 were not significantly different between the two groups. Between the group with *Plasmodium* only and the group with co-infection of *Plasmodium* and helminths, there were no significant differences in the levels of the AICs: IL-4, IL-10 and IL-13 (Table 4.32). In the group with co-infection of *Plasmodium* and HIV, concentration of IL-10 was significantly higher than those with *Plasmodium* only. The concentrations of IL-4 and IL-13 were not statistically different between these groups (Table 4.32).

Similar to the second trimester, median concentration of IL-13 in late stage of gestation was significantly higher in those with *Plasmodium* only relative to the uninfected. The levels of the remaining AICs, IL-4 and IL-10 were not significantly different (Table 4.32). In comparison with the group with co-infection of *Plasmodium* and helminths, the concentrations of all the AICs was not significantly different among those with *Plasmodium* only (Table 4.32). Among those with *Plasmodium* only, the level of IL-10 was significantly lower relative to those with co-infection of *Plasmodium* and HIV. The levels of IL-4 and IL-13 were not significantly different between these groups (Table 4.32).

There was no significant variation in mid and late gestational stages profiles of these cytokines in the different groups.

Table 4.31: Median (IQ) values of AIC concentrations of those with *Plasmodium* and its co-infection with helminths or HIV

	PF only (n=22)	PF + H (n=5)	PF + HIV (n=20)	Uninfected (n=29)	*P*- value
IL-4 (pg/mL)	8.936 (6.602-18.930)	10.106 (7.772-51.622)	10.430 (5.822-20.884)	8.352 (4.688-14.506)	
IL-10 (ng/mL)	0.097 (0.059-0.167)[a]	0.096 (0.018-0.175)	0.229 (0.135-0.346)[a]	0.126 (0.083-0.190)	0.001[a]
IL-13 (pg/mL)	127.428 (127.428-515.762)[b]	8.324 (8.324-161.522)	454.158 (113.919-1603.184)	<8.000[b]	p<0.0001[b]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.32: Median (IQ) values of AIC concentrations of those in second and third trimesters with *Plasmodium* and its co-infection with Helminths or HIV

Second trimester	**PF only (n=13)**	**PF + H (n=3)**	**PF + HIV (n=8)**	**Uninfected (n=14)**	***P*- value**
IL-4 (pg/mL)	9.521 (5.729-17.160)	7.772 (5.438-7.772-)	2.958 (0.094-2.958)	6.602 (3.986-18.964)	
IL-10 (ng/mL)	0.102 (0.064-0.172)[a]	0.019 (0.016-0.019)	0.235 (0.172-0.408)[a]	0.122 (0.088-0.186)	0.025[a]
IL-13 (pg/mL)	127.428 (127.428-373.259)[b]	161.522 (154.660-161.522)	964.203 (275.634-964.203)	<8.000[b]	p<0.0001[b]
Third trimester	**PF only (n=7)**	**PF + H (n=2)**	**PF + HIV (n=12)**	**Uninfected (n=10)**	***P*- value**
IL-4 (pg/mL)	7.185 (6.602-17.472)	42.358 (10.106-42.358)	12.448 (8.124-21.389)	8.352 (4.398-19.591)	
IL-10 (ng/mL)	0.074 (0.044-0.157)[a]	0.151 (0.096-0.151)	0.224 (0.103-0.330)[a]	0.145 (0.096-0.298)	0.011[a]
IL-13 (pg/mL)	210.002 (210.002-210.002)[b,c]	8.324 (8.324-8.324)[b]	454.158 (113.919-1542.712)	<8.000[c]	0.037[b]; 0.003[c]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus; Similar superscripts show significant difference at $\alpha_{0.05}$

4.6.5 Anti-inflammatory cytokine (AIC) profile modification in response to Helminth and its co-infection with *Plasmodium* or HIV

Among those with helminths only, the median concentration of IL-13 was significantly higher while IL-10 was significantly lower relative to the uninfected. IL-4 was not significantly different between the two groups. However, comparison of the group with helminths only and those with co-infection of *Plasmodium* and helminths showed that the levels of all the AICs were not significantly different (Table 4.33). Similarly, the levels of all the AICs: IL-4, IL-10 and IL-13 were not significantly different between those with helminths only and those with co-infection of helminths and HIV (Table 4.33).

In the second trimester, median concentration of IL-13 was significantly higher while IL-10 was significantly lower among those with helminths only relative to the uninfected while IL-4 was not significantly different (Table 4.34). Comparison of the group with helminths only and the group with co-infection of *Plasmodium* and helminths showed there were no significant differences in the concentrations of the AICs: IL-4, IL-10 and IL-13 (Table 4.34). For the group with co-infection of helminths and HIV, significant differences in concentrations of cytokines relative to the helminth only group could not be determined as there was a single sample of helminths and HIV co-infection (Table 4.34).

In the third trimester, level of IL-13 was significantly higher in the group with helminths only relative to the uninfected. The levels of the remaining AICs, IL-4 and IL-10 were not significantly different between the two groups (Table 4.34). In comparison to the group with co-infection of *Plasmodium* and helminths, median level of IL-13 was significantly higher among those infected with helminths only. IL-4 and IL-10 values were not significantly different between the two groups (Table 4.34). Relative to those with combined infections of helminths and HIV, the median concentrations of the AICs among those infected with helminths only was not significantly different (Table 4.34).

Furthermore, there was no significant variation in mid and late stage pregnancy profiles of these cytokines in the different groups.

Table 4.33: Median (IQ) values of AIC concentrations of those with Helminth and its co-infection with *Plasmodium* or HIV

	H only (n=21)	PF + H (n=5)	H + HIV (n=4)	Uninfected (n=29)	*P*- value
IL-4 (pg/mL)	12.448 (7.185-17.747)	10.106 (7.772-51.622)	13.902 (8.124-13.902)	8.352 (4.688-14.506)	
IL-10 (ng/mL)	0.077 (0.053-0.111)[a]	0.096 (0.018-0.175)	0.042 (0.039-0.072)	0.126 (0.083-0.190)[a]	0.008[a]
IL-13 (pg/mL)	87.664 (34.320-281.373)[b]	8.324 (8.324-161.522)	814.376 (150.729-814.376)	<8.000[b]	p<0.0001[b]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.34: Median (IQ) values of AIC concentrations of those in second and third trimesters with Helminth and its co-infection with *Plasmodium* or HIV

Second trimester	H only (n=13)	PF + H (n=3)	H + HIV (n=1)	Uninfected (n=14)	*P*- value
IL-4 (pg/mL)	11.276 (7.769-16.573)	7.772 (5.438-7.772-)	8.124 (8.124-8.124)	6.602 (3.986-18.964)	
IL-10 (ng/mL)	0.065 (0.053-0.077)[a]	0.019 (0.016-0.019)	0.039 (0.039-0.039)	0.122 (0.088-0.186)[a]	0.011[a]
IL-13 (pg/mL)	87.664 (34.320-260.896)[b]	161.522 (154.660-161.522)	1616.299 (1616.299-1616.299)	<8.000[b]	p<0.0001[b]
Third trimester	**H only (n=7)**	**PF + H (n=2)**	**H + HIV (n=3)**	**Uninfected (n=10)**	***P*- value**
IL-4 (pg/mL)	14.8 (4.276-18.338)	42.358 (10.106-42.358)	114.739 (13.902-114.739)	8.352 (4.398-19.591)	
IL-10 (ng/mL)	0.103 (0.049-0.111)	0.151 (0.096-0.151)	0.045 (0.039-0.045)	0.145 (0.096-0.298)	
IL-13 (pg/mL)	34.320 (34.320-368.593)[a,b]	8.324 (8.324-8.324)[a]	482.553 (150.729-482.553)	<8.000[b]	0.012[a] 0.001[b]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

4.6.6 Anti-inflammatory cytokine (AIC) profile modification in response to HIV infection and its co-infection with *Plasmodium* or Helminths

The median concentrations of IL-4 and IL-13 were significantly higher and IL-10 was significantly lower among those infected with HIV only relative to the uninfected (Table 4.35). However, in comparison to the group with co-infection of *Plasmodium* and HIV, the concentration of IL-4 was significantly higher while IL-10 was significantly lower among those with HIV only. The difference in concentration of IL-13 was not statistically significant between the two groups (Table 4.35). Relative to those with combined infections of helminths and HIV, only the level of IL-10 was significantly lower in those with HIV only. The differences in concentrations of IL-4 and IL-13 were not statistically significant between these groups (Table 4.35).

In the second trimester, median concentrations of IL-13 was significantly higher while IL-10 was significantly lower among those infected with HIV only relative to the uninfected. The concentration of IL-4 was not significantly different (Table 4.36). In comparison to those with co-infection of *Plasmodium* and HIV, the concentration of IL-4 was significantly higher while IL-10 was significantly lower among those with HIV only. The level of IL-13 was not significantly different between the two groups (Table 4.36). For the group with co-infection of helminths and HIV, significant differences in concentrations of cytokines relative to the helminth only group could not be determined as there was a single sample of helminths and HIV co-infection (Table 4.36).

In the late stage of pregnancy, median levels of IL-4 and IL-13 were significantly higher while IL-10 was significantly lower among those infected with HIV only relative to the uninfected (Table 4.36). In comparison with the group with co-infection of *Plasmodium* and HIV, the concentration of IL-4 was significantly higher while IL-10 was significantly lower among those with HIV only. The concentration of IL-13 was not significantly different between the two groups (Table 4.36). Relative to those with combined infections of helminths and HIV, only the concentration of IL-10 was significantly lower in those with HIV only. The concentrations of IL-4 and IL-13 were not significantly different between these groups (Table 4.36).

There was no significant variation in mid and late pregnancy profiles of these cytokines in the different groups.

Table 4.35: Median (IQ) values of AIC concentrations of those with HIV and its co-infection with *Plasmodium* or Helminths

	HIV only (n=29)	PF + HIV (n=20)	H + HIV (n=4)	Uninfected (n=29)	*P*- value
IL-4 (pg/mL)	20.884 (13.324-32.650)[a,d]	10.430 (5.822-20.884)[a]	13.902 (8.124-13.902)	8.352 (4.688-14.506)[d]	0.007[a] p<0.0001[d]
IL-10 (ng/mL)	0.003 (0.003-0.025)[b,c,e]	0.229 (0.135-0.346)[b]	0.042 (0.039-0.072)[c]	0.126 (0.083-0.190)[e]	p<0.0001[b,e] 0.003[c]
IL-13 (pg/mL)	567.011 (239.221-1227.874)[f]	454.158 (113.919-1603.184)	814.376 (150.729-814.376)	<8.000[f]	p<0.0001[f]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

Table 4.36: Median (IQ) values of AIC concentrations of those in second and third trimesters with HIV and its co-infection with *Plasmodium* or Helminths

Second trimester	HIV only (n=8)	PF + HIV (n=8)	H + HIV (n=1)	Uninfected (n=14)	*P*- value
IL-4 (pg/mL)	16.224 (11.584-28.511)[a]	2.958 (0.094-2.958)[a]	8.124 (8.124-8.124)	6.602 (3.986-18.964)	0.036[a]
IL-10 (ng/mL)	0.012 (0.003-0.025)[b,c]	0.235 (0.172-0.408)[b]	0.039 (0.039-0.039)	0.122 (0.088-0.186)[c]	0.002[b] p<0.0001[c]
IL-13 (pg/mL)	980.216 (299.075-1566.634)[d]	964.203 (275.634-964.203)	1616.299 (1616.299-1616.299)	<8.000[d]	p<0.0001[d]
Third trimester	**HIV only (n=20)**	**PF + HIV (n=12)**	**H + HIV (n=3)**	**Uninfected (n=10)**	***P*- value**
IL-4 (pg/mL)	24.397 (15.062-37.093)[a,c]	12.448 (8.124-21.389)[a]	114.739 (13.902-114.739)	8.352 (4.398-19.591)[c]	0.017[a] 0.008[c]
IL-10 (ng/mL)	0.003 (0.003-0.012)[b,d,f]	0.224 (0.103-0.330)[b]	0.045 (0.039-0.045)[f]	0.145 (0.096-0.298)[d]	0.001[b] 0.003[d] 0.045[f]
IL-13 (pg/mL)	563.532 (207.435-1086.327)[e]	454.158 (113.919-1542.712)	482.553 (150.729-482.553)	<8.000[e]	p<0.0001[e]

Key: IQ – Interquartile range, PF – *P. falciparum*, H – Helminth, HIV – Human Immunodeficiency Virus
Similar superscripts show significant difference at $\alpha_{0.05}$

4.7 RELATIONSHIP IN PARASITE DENSITY, ANAEMIA, IRON STATUS AND CYTOKINES

Relationship in parasite count, anaemia, iron status and cytokines were tested in the different groups. However, two groups were exempted from the analysis of correlation because of their small number of positive samples; *Plasmodium* and helminth combined infections group and helminth and HIV co-infection group. In the other groups, correlations between variables with sample size <10 were discarded.

Among those infected with *Plasmodium* only, there were strong positive correlations among some cytokines: TNF-α and IL-6 (r=0.668, $p<0.01$), IL-1α and IL-10 (r=0.566, $p<0.01$), IL-1α and IL-6 (r=0.702, $p<0.01$), IL-2 and IL-4 (r=0.505, $p<0.05$), IL-17 and IL-2 (r=0.647, $p<0.01$), IL-10 and IL-6 (r=0.519, $p<0.05$), IL-4 and IL-17 (r=0.508, $p<0.05$) as shown in Table 4.37. There was also a strong positive correlation between STf and IL-13 (r=0.546, $p<0.05$). In the trimester-based analysis, there were also significant correlations in the second trimester: IL-6 and IL-10 (r=0.557, $p<0.05$), IL-17 and IL-2 (r=0.714, $p<0.01$), IL-6 and IL-1α (r=0.756, $p<0.01$), IL-6 and IFN-γ (r=0.555, $p<0.05$), IL-10 and IFN-γ (r=0.584, $p<0.05$), Serum ferritin (SF) and malaria parasite (MP) count (r=0.622, $p<0.05$) (Table 4.38).

SF and IL-2 were negatively correlated (r=-0.535, $p<0.05$) among those infected with helminths. However, there were strong positive relationships between IL-4 and IL-17 (r=0.515, $p<0.05$), IL-6 and IL-10 (r=0.519, $p<0.05$) (Table 4.39). The sample sizes for trimester analysis were too low thus they were excluded.

Infection with HIV only showed negative correlations between viral load (VL) and CD4 count (r=-0.564 $p<0.01$), IFN-γ and IL-13 (r=-0.664, $p<0.05$), STf and IL-6 (r=-0.686, $p<0.05$). Details are shown in Table 4.40. The negative correlation between VL and CD4 was also observed among those in third trimester (r=-0.587, $p<0.01$) (Table 4.41).

Among those with co-infection of *Plasmodium* and HIV, there were negative correlations between VL and CD4 (r=-0.812, $p<0.01$), VL and IL-4 (r=-0.650, $p<0.01$), PCV and TNF-α (r=-0.744, $p<0.01$). However, IFN-γ and VL were positively correlated (r=0.670, $p<0.05$). Similarly, there was direct correlation between CD4 and IL-4 (r=0.540, $p<0.05$) (Table 4.42). Those in third trimester had positive correlations between MP and IL-6 (r=0.654, $p<0.005$), VL and IFN-γ (r=0.694, $p<0.05$) while the negative correlations observed were IFN-γ and MP (r=0.704, $p<0.05$), CD4 and VL

(r=-0.786, p<0.01), IL-4 and VL (r=-0.602, p<0.05), PCV and TNF-α (r=-0.786, p<0.01) (Table 4.43).

Among those with no infection, there were positive correlations between IL-2 and serum transferrin (STf) (r=0.573, p<0.05), IL-1α and IL-6 (r=0.595, p<0.01) (Table 4.44). In the second trimester, positive correlations were found between PCV and IL-17 (r=0.593, p<0.05), STf and IL-12(p70) (r=0.674, p<0.05), STf and IL-2 (r=0.813, p<0.01), IL-6 and IL-1α (r=0.768, p<0.01) (Table 4.45). The sample sizes for third trimester analysis were too low thus they were excluded.

Table 4.37: Correlation coefficient values between *Plasmodium* infection alone, anaemia, iron status and cytokines.

	MP	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12 (p70)	1L-13	IL-17
MP	1.000	-.203	.183	-.112	.257	-.082	-.195	-.057	-.148	.002	.156	.112	.036	-.012
PCV		1.000	-.155	-.186	-.367	.077	-.008	.047	-.056	-.222	-.085	-.070	.345	-.202
SF			1.000	-.041	-.174	-.029	-.503	-.267	-.368	-.096	-.059	-.343	-.165	-.147
STf				1.000	-.303	-.345	.235	-.257	.033	-.270	-.452	.063	**.546***	.093
TNF-α					1.000	.473*	.417	.239	.593	**.668****	.356	.134	.342	-.034
IFN-γ						1.000	.154	.351	.322	.343	.320	-.121	.342	.193
IL-1α							1.000	-.231	.309	**.702****	**.566****	-.252	.066	-.385
IL-2								1.000	**.505***	.017	-.034	-.055	-.123	**.647****
IL-4									1.000	.630	.228	.062	.259	**.508***
IL-6										1.000	**.519***	-.295	.137	-.172
IL-10											1.000	-.218	-.332	-.340
1L-12 (p70)												1.000	.091	-.199
1L-13													1.000	.060
IL-17														1.000

Key: MP – malaria parasite count, PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$, **significant at $p<0.01$

Table 4.38: Correlation coefficient values in second trimester between *Plasmodium* infection alone, anaemia, iron status and cytokines.

	MP	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12 (p70)	1L-13	IL-17
MP	1.000	-.497**	**.622***	-.221	.402	-.006	-.448	-.336	-.228	.039	.266	.123	.173	-.006
PCV		1.000	-.270	-.019	-.518	.053	-.040	-.231	-.068	-.183	-.327	-.054	.407	-.216
SF			1.000	.011	-.048	-.192	-.419	-.413	-.484	-.036	.095	-.337	-.117	-.143
STf				1.000	-.414	-.131	-.162	-.051	-.149	-.418	-.786*	-.025	.437	.021
TNF-α					1.000	.541	.153	.100	.609	.551	.393	.180	.378	-.264
IFN-γ						1.000	.294	.085	.425	**.555***	**.584***	-.150	.434	.123
IL-1α							1.000	.136	.636	**.756****	.445	-.378	.030	-.164
IL-2								1.000	.549	.110	-.018	-.004	-.267	**.714****
IL-4									1.000	.743	.468	.017	.082	.441
IL-6										1.000	**.557***	-.218	.291	-.211
IL-10											1.000	-.179	-.394	-.360
1L-12 (p70)												1.000	.016	-.258
1L-13													1.000	-.125
IL-17														1.000

Key: MP – malaria parasite count, PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$, **significant at $p<0.01$

Table 4.39: Correlation coefficient values between helminth infection only, anaemia, iron status and cytokines.

	AL	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12 (p70)	1L-13	IL-17
AL	1.000	-.002	-.224	.243	.187	-.383	-.255	.181	.107	-.201	-.006	.075	-.227	.044
PCV		1.000	.161	.373	-.166	-.233	.229	-.091	-.120	.082	.091	.308	-.151	-.316
SF			1.000	-.116	.775*	.045	.197	**-.535***	-.384	.286	-.414	.183	.083	-.297
STf				1.000	-.378	-.757*	.729	-.072	.102	-.250	-.631	.074	-.215	-.453*
TNF-α					1.000	-.282	-.247	-.299	-.468	.357	.129	-.793*	.264	-.321
IFN-γ						1.000	-.272	.092	-.045	-.183	.113	-.223	.180	.181
IL-1α							1.000	-.348	-.453	.446	.032	.433	-.288	-.662
IL-2								1.000	.161	-.482	-.262	.041	.035	.356
IL-4									1.000	-.429	-.198	.177	.063	**.515***
IL-6										1.000	**.519***	-.643	.324	-.400
IL-10											1.000	-.468	.364	-.083
1L-12 (p70)												1.000	-.270	-.211
1L-13													1.000	-.180
IL-17														1.000

Key: AL – *A. lumbricoides* egg count, PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$

Table 4.40: Correlation coefficient values between HIV infection only, anaemia, iron status and cytokines.

	VL	CD4	PCV	SF	SI	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12	1L-13	IL-17
VL	1.000	**-.564****	.040	-.152	.382	-.156	.204	.053	-.093	-.046	-.216	.373	.214	-.168	-.228	-.026
CD4		1.000	.253	.032	-.560	-.094	-.021	-.198	.085	.004	.108	-.057	-.416	.058	-.043	.034
PCV			1.000	.117	-.095	-.029	.085	-.282	-.052	-.019	.035	.077	-.035	-.110	.012	-.099
SF				1.000	.949	1.000	.400	.258	-.632	1.000	1.000	.211	.400	.	-1.000	1.000
SI					1.000	1.000	.866	.500	-.866	-1.000	1.000	.500	.866	1.000	-1.000	1.000
STf						1.000	-.322	.235	-.393	-.082	.203	**-.686***	-.564	.009	-.034	-.034
TNF-α							1.000	.125	.086	-.363	.182	.430*	.331	-.087	.032	.148
IFN-γ								1.000	-.288	-.199	.422	.109	.001	-.280	**-.664***	.045
IL-1α									1.000	.326	.044	.011	.354	-.091	.218	-.308
IL-2										1.000	.233	-.189	-.291	.215	.461*	-.056
IL-4											1.000	-.254	-.351	.244	.090	.383*
IL-6												1.000	.103	.309	-.396	.110
IL-10													1.000	-.280	-.142	-.026
1L12														1.000	.177	.252
1L-13															1.000	-.100
IL-17																1.000

Key: VL – Viral load, PCV – packed cell volume, SF – serum ferritin, SI – serum iron, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$, **significant at $p<0.01$

Table 4.41: Correlation coefficient values in third trimester between HIV infection only, anaemia, iron status and cytokines.

	VL	CD4	PCV	SF	SI	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12	1L-13	IL-17
VL	1.000	**-.587****	-.167	1.000		-.146	-.024	.082		-.218	-.343	.602	.436	-.133	-.170	-.084
CD4		1.000	.408	-.1000		.014	.683	-.436		.232	.203	-.265	-.218	.140	.071	.077
PCV			1.000	-.341	.089	.132	.406	-.778		-.296	-.167	-.232	.372	-.092	-.007	-.097
SF				1.000	.091											
SI					1.000											
STf						1.000	-.323	.464		.082	.268	-.675	-.514	-.192	-.182	-.291
TNF-α							1.000	-.220		-.255	.180	.176	.172	.166	.383	.299
IFN-γ								1.000		-.058	.682	-.083	-.429	-.283	-.682	-.109
IL-1α																
IL-2										1.000	.302	-.071	-.531	.351	.489*	.132
IL-4											1.000	-.157	-.436	.104	.099	.393
IL-6												1.000	.276	.417	-.133	.289
IL-10													1.000	-.216	-.047	.203
1L12														1.000	.229	.172
1L-13															1.000	-.035
IL-17																1.000

Key: VL – Viral load, PCV – packed cell volume, SF – serum ferritin, SI – serum iron, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$, **significant at $p<0.01$

Table 4.42: Correlation coefficient values between *Plasmodium* and HIV co-infection, anaemia, iron status and cytokines.

	MP	VL	CD4	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-13	IL-17
MP	1.000	-.481	.209	.005	-.049	.082	-.324	-.332	.070	-.007	.345	.212	.499*	-.096	.091
VL		1.000	**-.812****	-.031	-.452	.090	-.105	**.670***	.118	.156	**-.650****	-.363	-.345	.094	-.327
CD4			1.000	.375	.286	-.037	.077	-.363	-.346	-.222	**.540***	.267	.127	-.332	.319
PCV				1.000	.178	-.223	**-.744****	-.104	.015	-.366	.044	-.400	-.304	.080	.248
SF					1.000	-.600	-.269	-.319	-.219	.250	.060	-.416	.438	.378	.731*
STf						1.000	-.049	.156	.353	-.321	.390	.012	-.236	-.247	-.289
TNF-α							1.000	-.103	-.040	.542	-.160	.394	.118	-.431	-.082
IFN-γ								1.000	-.178	-.032	-.170	-.326	-.118	.279	-.341
IL-1									1.000	-.043	-.349	.341	.127	-.566	-.041
IL-2										1.000	-.086	-.237	.276	-.222	-.053
IL-4											1.000	-.180	-.205	-.068	-.161
IL-6												1.000	.375	-.168	.132
IL-10													1.000	-.080	.507
1L-13														1.000	-.008
IL-17															1.000

Key: MP – malaria parasite count, VL – Viral load, PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$, **significant at $p<0.01$

Table 4.43: Correlation coefficient values in third trimester between *Plasmodium* and HIV co-infection, anaemia, iron status and cytokines.

	MP	VL	CD4	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-13	IL-17
MP	1.000	-.550	.195	.046	.009	-.251	.153	**-.704***	.151	.149	.148	**.654***	.375	-.015	.341
VL		1.000	**-.786****	.120	-.257	.318	-.359	**.694***	.212	.218	**-.602***	-.305	-.430	.087	-.306
CD4			1.000	.279	.086	-.345	.401	-.319	-.600	-.210	.464	.085	.164	-.363	.284
PCV				1.000	-.107	-.304	**-.786****	.462	-.032	-.355	.041	-.437	-.499	-.018	.205
SF					1.000	-.400	-.029	-.232	-.143	.300	.348	-.116	.200	.564	.580
STf						1.000	.142	-.226	.300	-.372	-.073	-.134	-.200	-.086	-.384
TNF-α							1.000	-.148	-.039	.400	.061	.252	.487	-.409	-.250
IFN-γ								1.000	-.196	-.159	-.135	.344	-.382	.372	-.427
IL-1									1.000	-.051	-.596	.440	.371	-.610	-.036
IL-2										1.000	-.596	.440	.371	-.128	-.255
IL-4											1.000	-.483	-.231	.138	-.254
IL-6												1.000	.447	.282	.300
IL-10													1.000	-.136	.523
1L-13														1.000	.007
IL-17															1.000

Key: MP – malaria parasite count, VL – Viral load, PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin. *significant at $p<0.05$, **significant at $p<0.01$

Table 4.44: Correlation coefficient values between no infection status, anaemia, iron status and cytokines.

	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12 (p70)	IL-17
PCV	1.000	.153	.138	-.117	.084	-.005	-.461*	-.226	-.294	.101	-.078	.391*
SF		1.000	-.302	-.866	-.500	.000	.082	-.017	.866	-.866	.039	-.507
STf			1.000	.	.	.	**.573***	.015	.	.	.360	-.043
TNF-α				1.000	.441*	.417*	.	.	.398*	.472*	.	.
IFN-γ					1.000	.265	.	.	.105	.360	.	.
IL-1α						1.000	.	.	**.595****	.461*	.	.
IL-2							1.000	.145	.	.	.224	-.360
IL-4								1.000	.	.	-.238	.163
IL-6									1.000	.036	.	.
IL-10										1.000	.	.
1L-12 (p70)											1.000	-.029
IL-17												1.000

Key: PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin.
*significant at p<0.05, **significant at p<0.01

Table 4.45: Correlation coefficient values in second trimester between no infection status, anaemia, iron status and cytokines.

	PCV	SF	STf	TNF-α	IFN-γ	IL-1α	IL-2	IL-4	IL-6	IL-10	1L-12 (p70)	IL-17
PCV	1.000	.201	-.141	-.247	.189	.006	-.450	-.113	-.339	.171	.087	**.593***
SF		1.000	-.429				-.211	-.600			-.493	-.429
STf			1.000	.	.	.	**.813****	.291	.	.	**.674***	-.160
TNF-α				1.000	.394	.393	.	.	.269	.342	.	.
IFN-γ					1.000	.063	.	.	-.047	.233	.	.
IL-1α						1.000	.	.	**.768****	.228	.	.
IL-2							1.000	.239	.	.	.292	-.006
IL-4								1.000	.	.	-.397	-.046
IL-6									1.000	.027	.	.
IL-10										1.000	.	.
1L-12 (p70)											1.000	.027
IL-17												1.000

Key: PCV – packed cell volume, SF – serum ferritin, STf – serum transferrin, TNF-α – tumor necrosis factor alpha, IFN-γ interferon gamma, IL – interleukin.

*significant at p<0.05, **significant at p<0.01

CHAPTER FIVE

DISCUSSION

5.1 Patients' demographic details

Seven hundred and seven pregnant women reporting for ante-natal care (ANC) for the first time were recruited in this study. A larger percentage (80.6%) of these women were within 20-34 years. Similar reports of a larger fraction of women at booking being within this age group were reported in studies carried out in Niger-Delta area of Nigeria (Ndidi and Oseremen, 2011) and South-west Nigeria (Oladokun *et al.* 2010). Furthermore, most of the women in this study presented late for booking as 45.6% of the women were in second trimester while 48.0% were in the third trimester. Although the 2016 WHO ANC model recommends the first ANC contact within the first 12 weeks of pregnancy (WHO, 2016), a number of studies have also reported late booking in women as observed in this study (Adekanle and Isawumi, 2008; Oladokun *et al.* 2010; Ndidi and Oseremen, 2011). Early ante-natal registration has been associated with reduced perinatal morbidity and mortality thus efforts need to be geared towards encouraging early seeking of ante-natal care (Oladokun *et al.* 2010).

5.2 Prevalence and pattern of infections

This study reports that *Plasmodium sp,* intestinal helminths and HIV remain infections of public health challenges in Nigeria with resultant effects on host physiological and immunological parameters. Some aspects of the pathogenesis of these infections were evaluated among expectant mothers in this research. The findings further substantiated the existence of these pathogens either singly or as co-infection among apparently healthy pregnant women with their quantifiable effects on susceptibility to anaemia, modification of iron status and changes in cytokine profile.

In this survey, the number of cases of *Plasmodium* infection was 16.1%. In related studies within the same city, 23.1% and 8.4% prevalence were reported (Akanbi *et al.* 2006; Falade *et al.* 2008). Another city, Lagos in Southwest Nigeria had at different

times, reported prevalence of 7.7% and 52% (Agomo *et al.* 2009; Raimi and Kanu, 2010). However, a more recent report from the northern part of Nigeria recorded 36.5% and 41.6% cases respectively (Bawa *et al.* 2014; Fana *et al.* 2015). Although a larger fraction of these researches were implemented using parturient women registered in ante-natal clinics, women recruited in this study were strictly those reporting for ante-natal for the first time. This may account for the wide difference in values. The finding of Agomo *et al.* (2009) supports this observation as they also recruited at booking recording a relatively low malaria cases of 7.7%. Variations in number of cases across the country may also be a reflection of the success or failure of government strategies towards reducing malaria transmission. This study also showed that there was no significant association between *Plasmodium* infection and age or gravidity. This corresponds with an earlier report that showed no association between malaria and age. However, they reported a significant association between gravidity and malaria (Boel *et al.* 2010).

This study also recorded a prevalence of 8.8% of helminth infection. A hospital-based survey executed in the same location previously recorded 43.4% cases (Alli *et al.* 2011). Studies implemented in Southeast Nigeria noted helminth prevalence range of 16.3% - 32.4% in pregnancy (Ozumba *et al.* 2005; Obiezue *et al.* 2013; Dimejesi *et al.* 2014). The significant difference in prevalence shows the likelihood of increased awareness and health education talks on soil-transmitted helminths. *Ascaris lumbricoides* was the predominant helminth found in this research. Some studies within Nigeria also reported similar findings in Enugu (Ozumba *et al.* 2005), Jos (Egwunyenga *et al.* 2001) and Ibadan (Alli *et al.* 2011). However, it was not the predominant species found in a research implemented in Malawi (Thigpen *et al.* 2011). In Nigeria, *Ascaris* has remained the predominant species (Ekundayo *et al.* 2007).

Cases of multiple infections of *Plasmodium,* helminths and HIV were also found in this study. The endemicity of infectious diseases in sub-Saharan Africa makes harbouring multiple pathogens a more likely occurrence in the region. While most studies on co-infection carried out in this study area were in children, the participants in this study were pregnant women. Among the recruited women, there were 1.1% cases of *Plasmodium* and helminths co-infection, 0.8% had co-infection of HIV and helminths while 7.4% had co-infection of *Plasmodium* and HIV. Interestingly, *Ascaris lumbricoides* was the only helminth found among those with co-infection. In 2001, a

study in Jos reported a prevalence of 45% combined infections of *Plasmodium* and helminths among pregnant women (Egwunyenga *et al.* 2001). Recently, some authors reported prevalence of 5% combined infections of the two pathogens in gravid women enrolled in an ante-natal clinic in Osogbo, Osun State, Nigeria (Ojurongbe *et al.* 2018). This reported low prevalence corroborates the finding of this study.

In this study, those infected with *Plasmodium* only had higher parasite density compared with those having co-infection with *Ascaris*. However, the difference was not significant and it is presumed that the low rate of *Plasmodium* and *Ascaris* co-infection reported here may account for the insignificant difference. This contradicts the findings of studies done in Ethiopia among clinic outpatients and in Nigeria among children. They reported increased parasite density among those with *Plasmodium* and helminths co-infection relative to those having *Plasmodium* only (Degarege *et al.* 2012; Adedoja *et al.* 2015). In another research work on pregnant women with *S. haematobium* co-infection, they had lower malaria parasite densities (Thigpen *et al.* 2011) as communicated here too. It appears that reduced malaria parasite density in helminth co-infection with *Plasmodium* is pregnancy-related as the differences in data may be due to the immunological influence of pregnancy. In addition, there was no relationship between *Plasmodium* and *Ascaris* infection in this academic work. This corroborates the data obtained in a work carried out among expectant mothers in Thai-Burmese border (Boel *et al.* 2010).

In HIV and helminth co-infection, there were higher CD4 counts and viral loads among those with co-infection of HIV and *Ascaris* relative to HIV only albeit the differences were not significant probably because of the small number of samples. However, a previous investigation conducted among adults living with HIV in Uganda showed a similar pattern at the time of enrolment (Brown *et al.* 2004). According to a review of their findings and that of similar works, it was proposed that combined infections of helminth and HIV may have no detrimental effect on HIV disease advancement (Brown *et al.* 2006). However, Modjarrad *et al.* (2005) is of the opinion that co-infection of HIV and helminths may have more influence on HIV-1 replication among those with large infestation of helminth eggs together with high concentration of plasma RNA. This supports the report of a study wherein there was an elevation of CD4 counts with a corresponding decrease in viral load after deworming of helminth infected HIV positive patients (Means *et al.* 2016).

As obtained in HIV and helminth co-infection, the viral load in those with co-infection of *Plasmodium* and HIV increased relative to those infected with HIV only. A study of HIV-infected adults in Malawi reported similar finding (Kublin *et al.* 2005). This may increase transmission of HIV and speed up HIV progression (Kublin *et al.* 2005). Moreover, there was a corresponding higher malaria parasite density in those with co-infection compared with those infected with *Plasmodium* only. This corroborates the findings of preceding works (Kublin *et al.* 2005; Thigpen *et al.* 2011; Berg *et al.* 2014). Increased susceptibility to malaria may be linked to the placental and systemic immunological modifications that occur during pregnancy (ter Kuile *et al.* 2004).

5.3 Risk factors for *Plasmodium* and helminth infections

Using the logistic regression analysis, HIV-infected expectant mothers had higher chances of having malaria than those without HIV infection. Similar finding was reported among expectant mothers in Uganda and Malawi (Woodburn *et al.* 2009; Thigpen *et al.* 2011). Compromised immunity in HIV infection may inform the increased susceptibility to malaria. Other parameters assessed for risk of malaria infection in this study such as living in unplastered mud wall houses and absence of screens on doors or windows and low CD4 count showed no statistical significance. However, a previous study reported that lack of window screens and torn nets were linked to malaria positivity (Adedotun *et al.* 2010). The outcome on use of screens on doors or windows in this study may be due to confounding factors like possession of torn window or door screen which the structured questionnaire did not address.

Assessment of the risk factors for helminth infection showed that residents of houses with pit latrines or no toilet facility were at higher risks of infection. Similar findings were obtained among HIV-infected adult cohort in Kenya (Walson *et al.* 2010). This work supports the report of earlier studies as common place of defaecation, pit latrine and use of potty were all risk factors for helminth infection. Poor sanitary practices strongly influence the spread of helminths (Gyawali *et al.* 2013). Data from this research also noted that those with HIV were less disposed to harbour intestinal helminths than those without infection. This corroborates the findings of some workers although their data was specific for hookworm infection (Woodburn *et al.* 2009). In a study conducted in Honduras, HIV-negative patients also harboured more parasitic

infections and showed a higher chance of harbouring *Giardia lamblia*, *A. lumbricoides* and *T. trichiura* (Lindo *et al.* 1998). HIV probably induces a difference in the immunological status of the host intestine making it unfavourable for *Ascaris* eggs (Brenchley *et al.* 2008). Other variables showed no likelihood of helminth infection. For instance, there was no odds of malaria infection among those infected with helminths. Similar finding among pregnant women was recently reported in a study in Osogbo, South-west Nigeria (Ojurongbe *et al.* 2018). However, this contradicts earlier reports of other studies that showed significant relationships in malaria and helminth infections (Yatich *et al.* 2009; Degarege *et al.* 2012; Ivan *et al.* 2013). The low number of samples of *Plasmodium* and helminth (*Ascaris*) combined infections reported here may account for the insignificant association between the pathogens. This present study also showed no odds of helminth infection among women with respect to age. A similar report was obtained in a survey carried out in Northern Nigeria. The likelihood of helminth infection among pregnant women was unaffected by differences in age group (Fuseini *et al.* 2009).

5.4 Anaemia prevalence, iron status and associated risks in pregnancy

Anaemia defined as PCV < 33% (WHO, 2011a) was present in 57.6% of the pregnant women recruited. This corroborates other works in Nigeria (Idowu *et al.* 2005; Agan *et al.* 2010; Olatunbosun *et al.* 2014), Ghana (Baidoo *et al.* 2010), Ethiopia (Getachew *et al.* 2012) and Kenya (Ouma *et al.* 2007; McClure *et al.* 2014). However, some other works conducted in Nigeria reported <30% cases of anaemia in pregnancy (Falade *et al.* 2008; Gwarzo and Ugwa, 2013; Melku *et al.* 2014).

5.4.1 Packed Cell Volume and Infections

Data showed that those with *Plasmodium* infection had a significantly lower PCV relative to the uninfected group. Some studies have also reported reduction in PCV values among those with *Plasmodium* (Agan *et al.* 2010; Ifeanyi *et al.* 2014). It is proven that destruction of red blood cells, a key feature of malaria pathogenesis will predispose to anaemia (Pradhan, 2009). Moreover, the relationship between *Plasmodium* infection and anaemia was significant. This supports an earlier report of a

strong association between them (Agan *et al.* 2010). Similar reports were obtained from Uganda and Kenya (Muhangi *et al.* 2007; Ouma *et al.* 2007; Ndyomugyenyi *et al.* 2008).

Similarly, PCV values were significantly lower among those infected with HIV relative to the uninfected. Some studies have also reported reduced PCV values in HIV infection (Muhangi *et al.* 2007; Melku *et al.* 2014). However, a survey in Cote d'Ivoire demonstrated significant increase in PCV level among expectant mothers infected with HIV and taking antiretroviral treatment relative to uninfected women (Bleyere *et al.* 2013). Although, about 80% of those with HIV in this research were on antiretroviral therapy, it does not support the report of Bleyere *et al.* (2013). Anaemia in those with HIV influences progression of the disease, quality of life and mortality rate (Volberding *et al.* 2004). A strong association existed also between anaemia, HIV and CD4 count.

The odds of anaemia were 1.6-fold and 3.1-fold increases in those infected with *Plasmodium* or HIV respectively. This supports a study carried out in South Africa showing increased odds of anaemia among those with these infections (Hoque *et al.* 2009). Similarly, other studies carried out in Ethiopia and Uganda reported HIV as an independent predictor of anaemia (Muhangi *et al.* 2007; Melku *et al.* 2014). Helminth infection had no relationship with anaemia in this study. In Tanzania, a survey of schoolchildren also reported a similar finding (Mazigo *et al.* 2010). However, a previous report from a study in Ethiopia showed significant changes in PCV values in *Plasmodium* and STH infections (Getachew *et al.* 2012). No association observed in this work is probably a reflection of the low intensity of helminth reported. This study reports very few cases of hookworm infection which is known to predominantly cause anaemia due to attachment to intestinal walls.

5.4.2 Serum Ferritin and Infections

Plasmodium infection resulted in more than double increase in serum ferritin (SF) levels. Similar findings of increase in SF in the presence of *Plasmodium* infection have been reported among pregnant women (Friis *et al.* 2001), children (Das *et al.* 1997) and apparently healthy adults (Odunukwe *et al.* 2000). Ferritin, being an acute phase protein increases in concentration during inflammation (WHO, 2011b). Evaluation of SF according to gestational stages showed there was more than double rise in ferritin levels in the mid-trimester among those with *Plasmodium* infection relative to the uninfected. Similar pattern was observed in the late trimester but the difference was not significant. Ferritin concentration reportedly decreases as pregnancy progresses (Obirikorang *et al.* 2015; Okamgba *et al.* 2017).

Intestinal helminths infection also caused significant elevation in SF levels relative to the uninfected. Although there is a deficit of data on SF levels among helminth infected pregnant women in literature, a study among school children showed reduced SF in those infected with hookworm (Adebara *et al.* 2011). Furthermore, SF and *Ascaris* infection showed no association in this present study. Similar finding has been reported in children (Aini *et al.* 2007; Adebara *et al.* 2011). According to some workers, hookworms and *Trichuris* seem to be the main causes of low SF (Nurdiati *et al.* 2001; Aini *et al*. 2007). Hookworms have a higher probability of being strongly associated to SF because of their migratory behaviour leaving many bleeding spots leading to intestinal blood losses (Foy and Kondi, 1960). In trimester-based assessment, intestinal helminths infection caused significant elevation in SF levels in second trimester relative to the uninfected group but the difference was insignificant in the third trimester. A similar trimester-based evaluation of SF in pregnancy showed reduced levels of SF from first trimester to third trimester and *Trichuris* was identified as a major cause of low SF (Nurdiati *et al.* 2001). *Trichuris* worm burden was very low in this study and this may account for the insignificant association observed in this report.

HIV infection also showed a significantly higher level of SF relative to the uninfected group. Similar finding was reported by some workers in a research work on parturient women in Cote d' Ivoire (Bleyere *et al.* 2013). However, Friis *et al* found no difference in SF among women with or without HIV. The gravid women recruited in

this study and those recruited in Cote d' Ivoire were on antiretroviral therapy. Antiretroviral therapy is listed as one of the reasons for altered iron metabolism (Bleyere *et al.* 2013). HIV infection also caused an elevation in SF levels in second and third trimesters and similar findings were reported by Kharb and his colleagues (Kharb *et al.* 2017). Increases in ferritin may be due to translocation of iron from circulating erythrocytes to the stores. This is related to advancement in HIV clinical status (Friis *et al.* 2001).

Furthermore, there was no significant association in SF and CD4 count of HIV infected expectant mothers in this study. Same report was obtained among expectant mothers in Malawi (Semba *et al.* 2001). Similarly, there was no association between SF and PCV values in this present work. This agrees with the finding of an investigation carried out in Tanzania. It was postulated that this may be because low haemoglobin portrays a severe state of iron deficiency than SF which resonates level of iron stores (Kupka *et al.* 2007).

This work has further substantiated the evidence of high cases of anaemia among parturient women and infection has been singled as the key cause of anaemia in this setting. With the removal of confounders, there was a low 7.1% of iron deficiency anaemia (IDA) while the prevalence of infection associated anaemia was 35.5%. Contrary to this finding, ID is the dominant source of anaemia in some settings (Aini *et al.* 2007; Baidoo *et al.* 2010). This low number of IDA cases showed that nutritional deficiency may not be a public health priority among the studied population in this present study. A source of concern is the asymptomatic presentation of malaria among these gravid mothers at the first ante-natal visit. Presently, there is no guideline for routine screening for malaria and helminths in ante natal clinics in healthcare facilities in Nigeria. Hence, there is likelihood of these women remaining untreated as the adopted intermittent presumptive treatment in pregnancy (IPTp) has recorded cases of poor compliance (Akinleye *et al.* 2009; Arulogun and Okereke 2012). Furthermore, the desired result of iron augmentation on reducing the burden of anaemia in parturition may be masked without concurrent treatment of underlying infection.

5.4.3 Serum Iron and Infections

Serum iron (SI) level was significantly lower among those infected with *Plasmodium* only relative to the uninfected. Contrary to this report, elevated values of SI was reported among *Plasmodium* infected expectant mothers (Eteng *et al.* 2010). Another investigation implemented in children reported reduced SI level among *Plasmodium* infected children but the difference was not significantly different from the uninfected group (Das *et al.* 1997). However, the finding of a study of malaria patients in Cameroon agrees with the findings of this present study. They found a significantly reduced concentration of SI in individuals infected with *Plasmodium* relative to the uninfected group (Gouado *et al.* 2008). Variation in serum iron level results among *Plasmodium* infected individuals may be linked to the intensity of parasitemia and/or lack of clinical symptoms. In co-infection cases, SI levels of those infected with *Plasmodium* only was significantly lower than those with co-infections of *Plasmodium* and HIV. Alterations in metabolism of iron in malaria may influence susceptibility to combined infections (Spottiswoode *et al.* 2014). Similarly, SI level was significantly reduced among those infected with *Plasmodium* only relative to those with co-infection of *Plasmodium* and HIV in the third trimester. The higher levels of SI obtained in co-infection of *Plasmodium* and HIV relative to *Plasmodium only* may be due to antiretroviral drugs usage among the HIV patients thus having a positive effect on iron metabolism. Patients on Highly Active Antiretroviral Therapy (HAART) have been demonstrated to have a higher level of serum iron (Obirikorang *et al.* 2016).

In this study, SI level was highest among those infected with helminths only though insignificant. A study among school children in Medan reports that low serum iron may not be linked to intestinal helminths infection (Arrasyid *et al.* 2017). This agrees with the finding reported in this work.

SI level also reduced among those infected with HIV only relative to the uninfected but the variation was not significant. This is in agreement with an earlier report on HIV seropositive and seronegative individuals (Manafa *et al.* 2016). However, this contradicts earlier findings of elevated SI among those with HIV (Banjoko *et al.* 2012; Bleyere *et al.* 2013). It is likely the toll of anaemia on HIV may predispose to lower levels of iron (Obirikorang *et al.* 2016).

5.4.4 Serum Transferrin and Infections

Relative to the uninfected group, serum transferrin (STf) concentration was not significantly altered among those with *Plasmodium* only and those not infected. Contrary to this report, increase in STf level among malaria positive infected women has been reported (Eteng *et al.* 2010). Probably, the difference in findings may be because of varied clinical presentations of malaria. The expectant mothers in the study reported by Eteng *et al.* had clinical symptoms of malaria as opposed to this study wherein they were asymptomatic. In the trimester-based assessment, the differences in STf values remained insignificant in second and third trimesters. Furthermore, median STf concentration of those infected with *Plasmodium* only was significantly higher than those with co-infection of *Plasmodium* and HIV. This remained significant in both mid and late trimesters. Although data on measures of STf in pregnancy among *Plasmodium* infected women is sparse, the low values of STf in *Plasmodium* and HIV infections is attributed to alteration in iron metabolism by HIV (Minchella *et al.* 2015).

Those infected with helminths only had serum transferrin (STf) levels significantly higher than those with co-infection of helminths and HIV. As stated earlier, withdrawal of iron from circulation by HIV affects transferrin levels (Minchella *et al.* 2015).

Relative to the uninfected group, HIV had significantly lower levels of serum transferrin (STf) and this remained same in the trimester analysis of both second and third trimesters. A similar report was documented for HIV-infected non-pregnant women (Manafa *et al.* 2016). HIV blocks release of iron from enterocytes and also redistributes the iron to storage sites such as tissues macrophages (Minchella *et al.* 2015). Hence, rise in serum transferrin occurs only after iron stores become functionally depleted (Alper *et al.* 2000).

5.5 Cytokine profile changes in pregnancy

In Nigeria, few studies have evaluated the changes in cytokine profile of pregnant women in response to infectious diseases. These studies concentrated on single infection and evaluated few cytokines (Nmorsi *et al.* 2010a; Nmorsi *et al.* 2010b; Adeoti *et al.* 2015). In this study, more cytokines and multiple infections were evaluated. In the trimester-based analysis, the number of samples obtained for the first trimester were very few hence the cytokine profile analysis for the first trimester was excluded.

5.5.1 Pro-inflammatory cytokines (PICs) in *Plasmodium* and its co-infection with helminths or HIV

In this study, the PIC profile was not significantly altered among those infected with *Plasmodium* only relative to the uninfected. Contrary to this finding, some workers found increased IFN-γ, IL-2, IL-6 and IL-12 among individuals with malaria particularly those with mild malaria compared with the uninfected (Prakash *et al.* 2006). Similarly, increased IFN-γ was also reported among pregnant women with acute or severe malaria relative to those uninfected (Nasr *et al.* 2014; Nmorsi *et al.* 2010a). However, these cytokines have been found to decrease as malaria severity reduced (Prakash *et al.* 2006). Nmorsi *et al.* also noted decrease in concentration of IL-6 (Nmorsi *et al.* 2010a). Hence, the variation in results in these previous investigations compared with this work may be linked to the difference in clinical presentations of malaria in the studied populations. The expectant mothers in this study had no clinical symptoms of malaria. Development of clinical signs has been linked to endemicity of the study area to malaria (Sinha *et al.* 2010). Hence, differences in outcomes for varied study populations is expected. In the trimester-based assessment, the PIC profile of those infected with *Plasmodium* only was also not significantly altered relative to those uninfected in both mid and third gestational stages. This supports the finding of a similar study among asymptomatic expectant mothers in their late gestational stage where no significant changes in PIC levels was reported (Wilson *et al.* 2010).

Co-infection of *Plasmodium* with intestinal helminths had elevated levels of IL-2 and reduced levels of IFN-γ relative to *Plasmodium* only. On the contrary, a previous study of acute malaria cases in Brazil found no difference in cytokine profile of those with

co-infection of intestinal parasites and *Plasmodium* in comparison with those infected with *Plasmodium* only (Sánchez-arcila *et al.* 2014). Similarly, no appreciable change in IFN-γ was reported in those with co-infection and those with *Plasmodium sp* only among hospital out-patients in Uganda (Bwanika *et al.* 2018). The differences in study population may possibly influence the variation in results. Since increase in these cytokines correlated with malaria severity (Prakash *et al.* 2006), decrease in concentration of IFN-γ among those with co-infection of *Plasmodium* and helminths observed in this present investigation indicates that helminth protects in co-infection with *Plasmodium*. Likewise, co-infection of *Plasmodium sp* and helminths had a significantly lower concentration of IFN-γ relative to *Plasmodium* only in the mid gestational stage. In the late stage of pregnancy, IL-2 was significantly lower in those with *Plasmodium sp* only relative to those with co-infection. As earlier stated, changes in these cytokines have been correlated with malaria severity (Prakash *et al.* 2006). Thus, decrease in concentration of IFN-γ among those with co-infection of *Plasmodium* and helminths in this present study suggest a protective role of helminth in co-infection with *Plasmodium* particularly in the second trimester.

Relative to combined infections of *Plasmodium* and HIV, those infected with *Plasmodium* only had significantly lower concentrations of interleukins 1α and 17. The role of IL-17 as reviewed by Binqing *et al.* (2014) discusses its implication in recurrent abortion and pre-eclampsia. Increased IL-17 has been reported in women with preeclampsia relative to the healthy pregnant women (Wallace *et al.* 2011). Hence, pregnant women with co-infection of *Plasmodium* and HIV may be at higher risk of preeclampsia. The pattern of cytokine changes in *Plasmodium* and HIV co-infection relative to *Plasmodium* only was the same in mid and late trimesters.

5.5.2 Pro-inflammatory cytokines (PICs) in helminth and its co-infection with *Plasmodium* or HIV

The cytokine profile of those infected with helminths was significantly higher in concentrations of TNF-α and IL-2 while the concentrations of IFN-γ was lower relative to the uninfected group. The profile remained same when the uninfected group were compared with those infected with *Ascaris* only. In a study of *A. lumbricoides* infected children in Nigeria, similar result of higher TNF-α concentration was reported (Nmorsi *et al.* 2010c). Assessment by gestational stages also showed significantly higher concentrations of TNF-α, IL-2 and IL-17 and significantly lower levels of IFN-γ in those infected with helminths only in the second trimester relative to the uninfected. Elevated levels of TNF-α has been associated to risk for recurrent foetal loss, hypertensive syndromes, foetal growth restriction and gestational diabetes mellitus (Moreli *et al.* 2012). Hence, the significant levels of TNF-α in the second trimester may be deleterious to foetal development. Although there is paucity of data on the mechanism of action of IL-17 in nematodes infection, its ability to influence immune modulatory effects in a cestode infection, alveolar *Echinococcus* has been reported (Lechner *et al.* 2012). In the third trimester, only IL-2 was significantly altered in those with helminth only relative to the uninfected. A similar finding of higher levels of IL-2 was reported in asthmatic patients infected with helminths (Arinola *et al.* 2014). IL-2 acts in synergy with IL-4 to stimulate growth of B cells (Baqai, 1996). This may explain the consistent elevated values of IL-2 observed in this study as helminths elicit a Th2 response.

In co-infection of *Plasmodium* with intestinal helminths, IL-2 was significantly higher compared with helminths only. However, in the trimester-based analysis, co-infection of *Plasmodium sp* and helminths had significantly lower levels of IFN-γ relative to helminths only in the mid stage of pregnancy. In the late gestational stage, IL-6 was significantly lower in those with helminth only relative to those co-infected with *Plasmodium*. Increased maternal IL-2 has been associated with foetal developmental delay while elevated levels of maternal IFN-γ has been closely linked to autism spectrum disorders in the foetus in a case-control investigation of pregnant women (Goines *et al.* 2011). Hence, those with co-infection of *Plasmodium* and helminths may be at a higher risk of delayed foetal growth compared with those with helminth only.

Combined infections of helminths and HIV had significantly higher TNF-α, IFN-γ, IL-17 but significantly lower IL-1α compared with those infected with helminths only. Co-infection of helminths and HIV showed undetectable levels of IL-12 (p70). TNF-α is an inflammatory cytokine and its uncontrolled increase have been implicated in obstetric complications (Moreli *et al.* 2012). Thus, HIV infection in helminth-infected pregnant women may increase obstetric complications. There were no remarkable variations in the concentrations of other inflammatory cytokines relative to helminth only in second trimester. This is because only one patient in second trimester had co-infection of helminths and HIV. Thus, adequate statistical inferences could not be deduced. However, in the third trimester, IFN-γ and IL-17 were significantly higher while IL-1α was significantly lower in those with co-infection of helminths and HIV relative to those infected with helminths only. These cytokines appear not to have a significant function in pathogenesis of helminth infection. Hence, their roles in HIV pathogenesis will be discussed later.

In this study, PIC profile showed a significant change in IFN-γ between second and third trimesters. A previous study had established that these cytokines reportedly increased or were not altered significantly between second and third trimesters (Ross *et al.* 2016). However, another study showed that IFN-γ levels reduced as pregnancy progressed (Aris *et al.* 2007). Helminth infestation appear to mildly affect the expected PIC profile changes at different trimesters.

5.5.3 Pro-inflammatory cytokines (PICs) in HIV and its co-infection with *Plasmodium* or helminths

The array of cytokines were distorted in HIV infection. Relative to the uninfected, the cytokine profile of those infected with HIV showed higher levels of TNF-α, IL-6 and IL-17 while there were significant reductions in concentrations of IL-1α and IL-12(p70). Assessment of the cytokine profile by trimester showed that the cytokine profile of those infected with HIV showed distortions in same cytokines in the second and third trimesters except for IL-12(p70) which was not significantly altered in the third trimester. Similar increase in TNF-α in both mid and late parturition was obtained in other studies of HIV infected pregnant women relative to the uninfected (Sachdeva *et al.* 2008; Richardson and Weinberg, 2011). TNF-α and IL-6 increased likewise in a study population of HIV infected non-pregnant individuals (Tudela *et al.* 2014).

Increased TNF-α has been linked to suppressed HIV-1 multiplication in monocytes in systemic circulation and alveolar macrophages (Lane *et al.* 1999). Similarly, elevated IL-6 secretion in HIV infected monocytes has been linked to HIV replication (Birx *et al.* 1990). HIV infected macrophages initiate secretion of IL-1α, 6 and TNF-α, key cytokines involved in HIV replication (Breen, 2002). Thus, the probability of spontaneous preterm birth is increased with higher levels of IL-6 (Ferguson *et al.* 2014). Furthermore, IL-12(p70) was significantly lower relative to the uninfected in the mid gestation stage only in this work. Contrary to the findings here, some workers reported increased concentration of IL-12(p70) in patients with acute HIV infection relative to the control that had undetectable level of IL-12(p70) (Byrnes *et al.* 2008). Similarly, two studies conducted among pregnant women showed increased levels of IL-12(p70) has pregnancy progressed (Curry *et al.* 2008; Holtan *et al.* 2015). The variation in the result of the work reported here compared with previous studies may be attributed to the heterogeneity of the individuals or populations recruited which may also influence the outcome. Moreso, higher concentration of IL-17 obtained in this study has been documented also among those with HIV (Nantawat *et al.* 2007). In an earlier investigation, IL-17 was suggested as a suitable biomarker in monitoring advancement of disease in HIV patients (Al-Nafea *et al.* 2017). It could be deduced that IL-17 secretion is initiated by HIV infection and may not be influenced by gestational stages of pregnancy.

Combined infections of HIV and *Plasmodium* had significantly higher concentrations of IL-1α relative to those with HIV only. Likewise, there was a significant reduction in concentrations of TNF-α, IL-6, IL-12(p70) and IL-17. *Plasmodium* infection may control the progression of HIV-1 by making T cells active or by releasing immune factors like TNF-α (Ned *et al.* 2005). Furthermore, reduced levels of TNF-α is essential for phagocytic activity and regulation of parasite densities (Clark *et al.* 1990). In an earlier research, IL-1α was significantly higher in women with preterm premature rupture of membrane, who were in labour and had positive microbial culture in amniotic fluid (Romero *et al.* 1992). A review article extensively detailed the significance of IL-1α in pre-term labour (Nadeau-Vallee *et al.* 2016). It could be deduced that increased IL-1α as obtained in this study may have negative implications on birth outcome among those with co-infection of *Plasmodium* and HIV. Furthermore, lower levels of IL-17 among those with co-infection of *Plasmodium* and

HIV in the third trimester may induce an adverse birth outcome as the cytokine has been reported to increase throughout pregnancy particularly in the third trimester in a research carried out among healthy expectant mothers. It was inferred to be probably involved in implantation and foetal development (Martinez-Garcia *et al.* 2010).

Combined infections of HIV and helminths showed significantly higher levels of IFN-γ compared with those infected with HIV only and undetectable levels of IL-12(p70) which remained same in the analysis by trimester. IFN-γ has been reported to hinder multiplication of HIV-1 (Alfano and Poli, 2005). Hence, co-infection of helminths with HIV may delay the onset of AIDS. There were no significant differences in the concentrations of the other inflammatory cytokines relative to HIV only in second trimester. This is because only one patient in second trimester had co-infection of helminths and HIV. Thus, adequate statistical inferences could not be deduced.

There was also a significant difference in TNF-α in both trimesters. An earlier report established a decline in the concentration of this cytokine as pregnancy progresses. However, the rationale for this is not yet certain (Ibitokou *et al.* 2015).

5.5.4 Anti-inflammatory cytokines (AIC) in *Plasmodium* and its co-infection with helminths or HIV

Since IL-13 was undetectable in the serum of the uninfected group, the cytokine was not considered during comparative data analysis of single infections and uninfected.

The AIC profile of those infected with *Plasmodium* only was not significantly altered relative to those uninfected. Assessing the AIC profile by trimesters, the cytokine profile of those infected with *Plasmodium* only was not significantly altered relative to those uninfected in both second and third trimesters. Contrary to this, earlier works showed higher levels of IL-10 relative to healthy control group in parturient women with no clinical malaria (Wilson *et al.* 2010; Megnekou *et al.* 2015). This variation in findings may be due to likely differences in the malaria parasite densities of the populations studied.

Co-infection of *Plasmodium* with intestinal helminths also showed no significant changes in these cytokines relative to *Plasmodium* only. This result was the same in the trimester-based analysis except in third trimester where concentration of IL-13 was

significantly lower in co-infection of *Plasmodium sp* and helminths relative to *Plasmodium* only. This outcome is in agreement with the works of Wilson *et al.* who found no significant difference in IL-10 in third trimester pregnant women with co-infection of *Plasmodium* and helminths relative to those with *Plasmodium* only (Wilson *et al.* 2010). However, a study among hospital out-patients in Uganda reported a highly significant difference in IL-10 among those with co-infection of *Plasmodium* and helminths and those with *Plasmodium sp* only (Bwanika *et al.* 2018). Pregnancy is connected to higher expression of IL-4 and IL-10 and may not be altered significantly in the presence of infection particularly when the parasite densities are quite low as reported here (Formby, 1995). More so, increased IL-10 has been reported among patients with cerebral malaria relative to those with acute cases of malaria (Prakash *et al.* 2006) thus linking this cytokine to malaria severity.

However, IL-10 levels was significantly elevated in those with co-infection of *Plasmodium* with HIV relative to those infected with *Plasmodium* only. This pattern of cytokine changes in *Plasmodium* and HIV co-infection relative to *Plasmodium* only were the same in second and third trimesters. Elevated concentrations of IL-10 in *Plasmodium* and HIV combined infections has been reported (Ibitokou *et al.* 2015). Increased IL-10 production in co-infection of *Plasmodium* and HIV has been termed an immunological response for protection against malaria and poor birth outcomes (Requena *et al.* 2015).

5.5.5 Anti-inflammatory cytokines (AICs) in helminth and its co-infection with *Plasmodium* or HIV

The cytokine profile of those infected with helminths only showed significantly lower IL-10 concentration and higher concentration of IL-13 relative to the uninfected. The profile remained same when data was analysed on trimester basis except in third trimester when IL-10 showed no significant difference. In a study of *A. lumbricoides* infected children in Nigeria, an increase in concentration of IL-10 contrary to this study was reported (Nmorsi *et al.* 2010c). This may be attributed to divergence in study population as intensity of *Ascaris* infection reduces with age (Turner *et al.* 2003). Similarly, low immunologic reaction is associated with chronic pathology and low

parasite burden (McSorley and Maizels, 2012). This is evident in this group of helminth positives only as 84.2% of them had *Ascaris* burden of <5000 eggs per gram.

In comparison to the group with co-infections of *Plasmodium* and helminths, there were no significant changes in AICs relative to the group with helminths only. The trimester-based analysis remained same except for IL-13 which was significantly lower in co-infection of *Plasmodium* and helminths relative to helminths only. The low helminth burden in this study may inform the insignificant changes in the cytokine profiles.

Relative to those with co-infections of helminths and HIV, there were no significant changes relative to those with helminths only. This was unaltered in the trimester-based analysis probably because of the small number of samples.

5.5.6 Anti-inflammatory cytokines (AICs) in HIV and its co-infection with *Plasmodium* or helminths

Relative to the uninfected, the cytokine profile of those with HIV had significantly higher levels of IL-4 and IL-13 but lower concentration of IL-10. Assessment by trimester revealed the same pattern except IL-4 that showed no significant difference in the second trimester. Elevated concentrations of IL-4 and IL-10 was reported by Richardson and colleague both in second and third trimesters (Richardson and Weinberg, 2011). In support of the findings of this study, IL-10 secretion was higher in cells derived from HIV-uninfected pregnant women relative to the pregnant women (Hygino *et al.* 2012). Although IL-10 was significantly lower in this study contrary to Richardson and colleague's report, a previous study showed that IL-10 significantly increased in HIV patients as disease progressed but reduced in those on highly active antiretroviral therapy (HAART) (Stylianou *et al.* 1999). The disparity in results may be due to the use of HAART by majority of the women recruited in this study. Furthermore, IL-10 also functions by terminating secretion of cytokines by activated macrophages to hinder a chronic inflammatory state from developing (Breen, 2002). Hence, HIV-infected pregnant women may be prone to complications if IL-10 concentration increases.

Co-infection of HIV and *Plasmodium* had significantly higher IL-10 and a significantly lower IL-4 relative to those infected with HIV only. According to a review, *Plasmodium* infection may regulate advancement of HIV-1 by T cells activation or secretion of TNF-α that could initiate the multiplication of HIV-1 (Ned *et al.* 2005). The pattern of cytokine changes in *Plasmodium* and HIV co-infection relative to the single infections were the same in second and third trimesters. This contradicts an earlier report of no significant changes in co-infection of *Plasmodium* and HIV and cases of HIV infection only among second trimester pregnant women (Ibitokou *et al.* 2015). However, elevated concentrations of IL-10 in *Plasmodium* and HIV combined infections has been reported (Ibitokou *et al.* 2015). Increased IL-10 production in co-infection of *Plasmodium* and HIV is an immunological response for protection against malaria and poor birth outcomes (Requena *et al.* 2015). HIV in pregnancy also supports increased expression of IL-10–producing CD4 T cells and is reported to lower the chances of vertical transmission of HIV-1 (Hygino *et al.* 2012).

IL-10 was significantly higher in co-infection of HIV and helminths compared with those infected with HIV only. This remained same in the third trimester. Increased IL-10 plays a part in damaged unacquired immune responses in those with AIDS (Ma and Montaner, 2000). Another research noted that the impairment of HIV immune profile by helminth infection occurs particularly in those with helminth eggs in their stools and high serum IgE (Mkhize-Kwitshana *et al.* 2011). Here, the gravid women were regarded as positive for helminth by the detection of worm eggs in the stool samples.

5.6 Correlations in cytokine profile and iron status indices

Possible correlations in cytokine profile and iron status was evaluated in the groups with single infections and the group with co-infection of *Plasmodium* and HIV. The groups with co-infection of *Plasmodium* and helminths and co-infection of helminths and HIV were excluded because of the small sample sizes.

In those with *Plasmodium* only, there was a direct relationship between STf and IL-13. Hence, as transferrin increases in circulation which is an indication of low levels of iron, IL-13 may enhance uptake and storage in activated macrophages (Weiss *et al.* 1997).

Among those infected with helminths only, serum ferritin (SF) and IL-2 were negatively correlated. This suggests cytokines may be involved in regulation of iron

transfer from tissues to blood (Lissoni *et al.* 1993). However, increased concentration of SF which has been reported in this study may negatively impact levels of IL-2 which is involved in initiation of Th2 response in helminth infection.

There was a direct association between IL-2 and serum transferrin ($r=0.573$, $p<0.05$) among those uninfected with *Plasmodium*, helminths or HIV. Transferrin is important for the development of IL-2 induced natural killer and natural killer-like activities (Shau *et al.* 1986).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions and recommendations

An overall assessment of the immunological effects of single and co-infections of *Plasmodium,* intestinal helminths and HIV in pregnancy has shown that malaria and HIV altered ferritin levels thus increasing the likelihood of iron deficiency among the pregnant women while HIV caused reduction in circulating levels of serum transferrin. Anaemia was highly prevalent in pregnancy and was aggravated by malaria and HIV infections. Helminth seems protective in combination with malaria or HIV having no profound impairment of the cytokine profiles. However, HIV and its co-infection with malaria greatly altered the pro-inflammatory cytokine profile in both second and third trimesters.

It is noteworthy that this research work was executed in Ibadan, a sub-urban city in the Southwest part of Nigeria. The outcome of this study is a predictor of a more deplorable state of the rural community as the cases of malaria and helminths in rural locations will most probably be much higher.

Recommendations

1. Malaria control should be intensified through continuous effective interventions like indoor residual spraying, environmental sanitation, subsidization of antimalarial drugs and sharing of insecticide- treated nets (ITNs) and long-lasting insecticide nets (LLINs) particularly in ante-natal clinics.
2. Good sanitary practices and adequate waste and sewage disposal channels will aid in elimination and eradication of helminths.
3. Ante-natal health talks addressing the importance of good sanitary practices like hand washing before preparing meals and not walking barefoot should be encouraged to reduce the number of helminthiasis cases.

4. The construction of houses with no toilet facilities should be discouraged by the relevant government agency as it has shown that this is a very strong contributor to spread of helminths. Helminths thrive in humid settings thus increasing the chances of infection when people defaecate on dung hills and open fields.
5. As a complement to deworming and use of IPTp, the routine screening for malaria and helminths at antenatal clinic visits for those infected with HIV and also those with PCV values <33% may extensively reduce the burden of anaemia.
6. Further research on the impact of treatment on number of cases of anaemia among gravid mothers with malaria parasitemia or helminthiasis will lay further credence to the need for routine screening during ante-natal visits.

6.2 Limitations of the study

In this present research, there were few cases of combined infections of *Plasmodium* and helminths among the expectant mothers. So also is co-infection of HIV and helminths. Hence, the capacity to make strong statistical inferences is reduced. More studies with larger number of co-infection cases will further increase the body of existing knowledge on impact of co-infection on immunological profile. Furthermore, assessment of other nutritional deficiencies, acute phase proteins and serum transferrin receptor would have enabled a more detailed explanation of the causes of anaemia.

6.3 Contributions to knowledge

This work has made available data on the current epidemiological status of three common diseases of poverty, prevalence of co-infections and the implications on parameters for determining disease severity among the parturient women recruited. The work has also provided additional information on effects of *Plasmodium,* intestinal helminths and HIV and the co-infections on maternal iron indices. Furthermore, the immunological interplay of these infections and the implications on the overall cytokine profile was assessed. The study also reported a trimester-based evaluation of these diseases.

The data showed that co-infection of infectious diseases impairs the cytokine profile of single infections. HIV and its co-infection with malaria greatly altered the PIC profile

in both mid and late stages of pregnancy. However, helminth seems protective in co-infection with malaria or HIV. This information is vital for vaccine and drug efficacy trials in regions of high endemicity of malaria and HIV combined infections as the efficacies of these products may be compromised.

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APPENDIX 1

Questionnaire

1. Study ID : IDEAP ...../..../..... 2. Hosp. No: .... 3. Date of enrolment: ....

4. Name: ....5. Age (yrs): ------------ 6. PCV (%)............

7. Date of birth ..../............./........../ 8. Weight (kg): .... 9. Height (cm): ....
dd mm yy

10. LMP: ---/------/------/ 11. Gravidity/-----/-----/ 12. Parity /----/----/ 13. Abortions /----/----/
dd mm yy

14. Temp (°C): .... 15. BP /--------/---------/ 16. No alive /------/---------/

17. Address (+ brief description): 18. HIV Status (+ve = 1, - ve = 2, don't know = 3) ☐

....

19. Level of education

SELF if>18 year: 1- None, 2- Primary/Quranic, 3 – Secondary, 4 – Post secondary ☐

Spouse/partner 1- None, 2- Primary/Quranic, 3 – Secondary, 4 – Post secondary ☐

20. Occupation

SELF if>18 years: 1- Student/unemployed, 2. petty trader
3 – Primary school teacher/ junior civil servant/artisan
4 –High school teacher/ middle level civil servant/middle business person
5 – Major Business person/ professional ☐

Spouse/partner : 1-- Student/unemployed, 2. petty trader
3 – Primary school teacher/junior civil servant/artisan
4 –High school teacher/ middle level civil servant/middle business person
5 – Major Business person/ professional ☐

21. Type of accommodation: 1 – Rented, 2- Family owned, 3 – Self, 4- Parent owned ☐

22Facility in accommodation:

i. Type of wall:

- 1 – Mud wall not plastered,
- 2 - Mud wall plastered,
- 3 – Cement block wall not plastered
- 4 – Cement wall plastered

☐

ii. Type of Toilet facility:

- 1 - None
- 2 - Pit latrine
- 3 - Water system

☐

2

iii. Availability of portable water in accommodation

- 1 - None
- 2 - Well with pump
- 3 - Well with bucket and rope
- 4 - Pipe borne water
- 5 - Borehole

☐

23. Helminthiasis history & treatment

i. How often do you wash your hands before food preparation and consumption? ☐

Never=1, Occasionally=2, Always=3 ☐

ii. Do you walk barefooted around the house, school or workplace? YES=1, NO=2

iii. When last did you take medicine to kill worms (Weeks)? ---------------------------

What is the name of the drug? ____________________

iv. Did you take any of the following drugs in the last 6 months?

i.	Ketrax	Yes / No
ii.	Combantrin	Yes / No
iii.	Antepar	Yes / No
iv.	Ivermectin	Yes / No
v.	Thiabendazole	Yes / No
vi.	Mebendazole	Yes / No

v. How often do you take worm medicine?

Weekly=1, Twice a week=2, Every two weeks=3,

Once a month=4, Once a Year=5, Others=6 ☐

vi. Where do you defecate more commonly?

i. Water closet	Yes / No
ii. Open field/Bush	Yes / No
iii. Dung hill near the house or school	Yes / No
iv. Pit latrine	Yes / No
v. Potty	Yes / No

vi. Others (Specify): ____________________

vii. When last did you pass worms (Weeks)? --

viii. What other symptoms did you observe?

Diarrhoea /water/stool=1, Abdominal pain=2, Fever=3,

Vomiting=4, Passed worm=5, Others (Specify) =6 ☐

ix. Have you received any health education on worm infection/its prevention in the last one year?

YES=1 NO=2 ☐

3

x. Do you use sewage/untreated faeces as fertilizer for your farm? YES=1, NO=2

xi. How can you prevent worm infections?

Drugs=1 Herbs=2 Others=3 (specify) ---------

24. OBSERVATION CHECKLIST

(Most responses needed in this section should be from the interviewer's observations of the household environs)

i. What can you say about the general sanitation of the house environ

Clean=1 Not clean=2 others=3 please specify ------------------------

ii. What material is the following parts of the house made of ?

The Roof __________, The Walls __________, The Floor __________

iii. Do the windows or doors have screens YES=1, NO=2

iv. Presence of potential breeding sites in the vicinity of the house (circle any appropriate option)

Clearing of vegetation=1, Close farm/cultivated land =2,

Fish pond=3 Others=4 (please specify) ----------------------

v. Presence of stagnant water YES=1 NO=2

vi. Are water collection containers covered? YES=1 NO=2

vii. Is there any open drainage system around the house? YES=1 NO=2

viii. How close is the stream/ river to the house?

Very far=1 Far=2 Close=3 Very close=4

ix. Nearness of toilet facility to main building

Very far=1 Far=2 Close=3 Very close=4

25. Present History

SYMPTOMS	D0	D1	D2	D3	D4	D7	D14	D21	D28
FEVER									
VOMITING									
CHILS & RIGORS									
DIARRHOEA									
COUGH									
HEADACHE									
ABDOMINAL PAINS									
LOSS OF APPETITE									
YELLOWNESS OF EYE									
OTHER COMPLAINTS									

4

26. Treatment history: Have you received any of the following drugs in the last 2 weeks YES / NO

Chloroquine ( ), Fansidar ( ), Paracetamol ( ), Antibiotic ( ) Artemisinin Specify ---------------- ACT: ( )

Herbal preparation: ------------------------ Worm expeller ( ), Specify if yes ----------------------

Are you currently on any opportunistic infection prophylactic therapy: Yes/No

If Yes specify -- Duration (Months) ---------------.

27. Antiretroviral therapy: Yes/No. If yes state combination --

--Duration (mths) ------------------------------

28. Clinical examination:

Date of examination: /----/-----/----------/

General: Palor (Y/N) Jaundice (Y/N)

Cardiovascular system: Pulse/min ------------- BP (mmHg) --------/---------

Lung fields: - Clear YES=1, NO=2

- Added sounds YES=1, NO=2
- Bronchial Breathing YES=1, NO=2
- Ronchi YES=1, NO=2
- Crepitations YES=1, NO=2

29. IRON STATUS CHECKLIST

i. Have you received any haematinics or other mineral supplements before reporting for antenatal care?

YES/NO

Folic acid ( ), B/Complex ( ), Ferrous sulphate ( ), Blood tonic ( ) Specify ....

ii. Any history of blood transfusion in the past 8 months? YES/NO When? ....

iii.Any history of vaginal bleeding? YES/NO

5

APPENDIX 2

Absolute CD4 Count Estimation

Five millilitres (5 mL) of whole blood was collected in Vacutainer bottles for Absolute CD4 count estimation.

Procedure

1. 20µL of CD4 monoclonal antibodies PE was added to 20µL whole blood in a Partec tube.
2. This was mixed gently and incubated for 15 min at room temperature in a dark room.
3. 800µL of no lyse buffer was then added and vortexed gently.
4. The sample was analysed using Flow Cytometry method.

APPENDIX 3

Viral Load Determination

RNA extraction

The extraction of RNA from plasma was done using HIV-1 Amplicon monitor which contains lysis buffer A and B, Master mix, Manganese ion, wash buffer, 96-well ELISA plate, hybridization reagent, conjugate, substrate A and B, dilute sulphuric acid, denaturation reagent, positive and negative controls for HIV.

Whole blood was centrifuged at 3000g for 30 min to obtain plasma.

1. 100µL of lysis buffer and 200µL of plasma was added in a cryovial. This was vortexed for thorough mixing.
2. The mixture was incubated for 10 min on the bench.
3. 100µL of isopropanol was added and vortexed.
4. The cryovials were transferred into micro-centrifuge and spun at 14,000 rpm for 30 min to concentrate the RNA.
5. The supernatant was removed using fine tip Pasteur pipette
6. 100µL of freshly prepared 30% ethanol was added and vortexed again.
7. The vials were centrifuged at 14,000 rpm for 15 min
8. The supernatant was removed and the vials blotted on absorbent paper.
9. 100µL of diluent was added and the vial scratched gently on sample rack to dislodge the RNA at the base of the tube.

DNA Amplification

1. Master mix was reconstituted by adding 100µL of Manganese to 100µL of Master mix.
2. 50µL of the reconstituted Master mix was aliquot into all the reaction (PCR) tubes including controls.
3. 50µL of extracted RNA was added into each tube.
4. The tubes were arranged in the Thermocycler and programmed.

5. RNA was transcribed to cDNA.
6. In the last 15 min, the amplicon from Thermocycler at 72°C was removed and 100µL of denaturation reagent was added.

Viral load count

1. In the detection room, 100µL of hybridization reagent was added to all the ELISA plate wells.
2. 50µL of amplicon was added to the wells and mixed 5 times.
3. 50µL from column A was serially diluted to column F.
4. 50µL from PCR tubes was serially diluted from column G to H (G and H are internal controls for all the samples including the negative, low positive and high positive controls).
5. The plate was sealed and incubated at 37°C for 1 hr.
6. The plate was washed five times using 400µL of wash buffer per well.
7. The plate was blotted on absorbent paper.
8. 100µL of conjugate was added and incubated for 15 min.
9. The plate was washed again as described above.
10. Substrate A and B was added together then 100µL of the reconstituted substrate was added into each well.
11. It was then incubated in the dark at room temperature for 10 min.
12. 100µL of Stop Solution was added to each well.
13. The optical density (OD) was read using the microplate reader at 650nm and 450nm to take care of background readings.

APPENDIX 4

Serum ferritin assay procedure

The enzyme immunoassay was carried out using pre-coated human enzyme-linked immunosorbent assay (ELISA) test kits obtained from Assaypro, USA. Standard curves were generated and data interpreted using Curve Expert 1.4 software.

Reagent preparation

1. The EIA Diluent was provided as a 10X concentrate. It was diluted to a concentration of 1X using distilled water.
2. Reconstitution of the 75ng of Human Ferritin Standard with 1.5mL of EIA Diluent produced a solution of 50ng/mL. This was allowed to sit for 10 min with gentle agitation before making the dilutions.
3. The standard solution was diluted 1:2 with same volume of EIA Diluent to make serial dilutions of 25, 12.5, 6.25, 3.125, 1.563 and 0.781 ng/mL solutions. EIA Diluent served as the zero standard (0 ng/mL).
4. Biotinylated Human Ferritin Antibody (100X) was spun down briefly using a centrifuge and diluted 1:100 with EIA Diluent.
5. Using distilled water, the Wash Buffer Concentrate (20X) was diluted to a concentration of 1X.
6. Streptavidin-Peroxidase (SP) Conjugate (100X) was spun down briefly and diluted 1:100 with EIA Diluent.

Assay procedure

1. 50µL of standard or sample was added into each microplate well which was then covered after the last sample had been added. It was then incubated for 2 hr.
2. The wells were manually washed five times using 200µL of Wash buffer per wash. The plate was inverted each time and the contents decanted; then blotted 4-5 times on an absorbent paper material in order to completely remove the liquid.
3. 50µL of Biotinylated Human Ferritin Antibody was added to each well and incubated for 1 hr.
4. The wash procedure was repeated again as described above.
5. 50µL of SP Conjugate was added to each well prior to incubation for 30 min.

6. The microplate was washed as described above.
7. 50µL of Chromogen Substrate was added into each well before incubation for 12 min. The microplate was tapped gently to ensure thorough mixing.
8. 50µL of Stop Solution was then added to each well with a noticeable colour change from blue to yellow.
9. Absorbance values were generated using a microplate reader at a wavelength of 450nm immediately.

Serum Ferritin values <12ng/mL was used as cut-off among those not infected. To compensate for inflammation, the cut-off was raised to 30ng/mL for those infected (WHO, 2011). Among those infected, iron deficiency anaemia was defined as PCV <33% and serum ferritin values <30ng/mL (WHO, 2007).

APPENDIX 5

Serum transferrin assay procedure

The enzyme immunoassay was carried out using pre-coated human ELISA test kits obtained from Assaypro, USA. Standard curves were generated and data interpreted using Curve Expert 1.4 software.

Reagent preparation

1. The MIX Diluent Concentrate (10X) was diluted to a 1X concentration using distilled water.
2. The 75µg of Human Transferrin Standard provided was reconstituted with 3mL of MIX Diluent to produce a 25µg/mL standard solution. Before making dilutions, the preparation was allowed to sit for 10 min with gentle agitations.
3. The standard solution was diluted 1:4 with MIX Diluent serially producing 6.25, 1.563, 0.391 and 0.098 µg/mL of standard solutions. MIX Diluent served as the zero standard (0 µg/mL).
4. Biotinylated Human Transferrin (6X) was reconstituted with 4mL MIX Diluent to produce a 6-fold stock solution which was further diluted 1:6 with MIX Diluent.
5. Wash Buffer Concentrate (20X) was diluted to 1X concentration using distilled water.
6. Streptavidin-Peroxidase (SP) Conjugate (100X) was spun down briefly using a centrifuge and diluted 1:100 with MIX Diluent.

Assay procedure

1. 25µL of standard or sample was added per well with 25µL of Biotinylated Human Transferrin added on top of each standard or sample. The plate was tapped to mix gently.
2. The wells were covered and incubated for 1 hr after the last sample addition.
3. The wells were manually washed five times with 200µL of Wash buffer. The plate was inverted per was and the contents decanted; then hit 4-5 times on absorbent paper material to completely remove the solution.
4. 50µL of SP Conjugate was added to each well prior to incubation for 30 min.
5. The wash procedure was repeated as described above.

6. 50µL of Chromogen Substrate was added per well with gentle tapping of the plate to ensure thorough mixing. The plate was incubated for 10 min..
7. 50µL of Stop Solution was added to each well and there was a noticeable colour change from blue to yellow.
8. Absorbance values were generated using a microplate reader at a wavelength of 450nm immediately.

APPENDIX 6

Determination of Human IFN-ɣ concentration

This was done using pre-coated Human ELISA kits obtained from Assaypro, USA. Samples were diluted 1:2 in distilled water before use after being brought to room temperature.

Reagent preparation

1. The EIA Diluent Concentrate (10X) was diluted 1:10 with distilled water.
2. Human IFN-ɣ Standard supplied was 2ng and this was reconstituted with 2mL of EIA Diluent to make 1ng/mL solution. This was allowed to sit for 10 min, gently agitated before dilutions were made.
3. The standard solution was diluted 1:2 with equal volume of EIA Diluent serially to produce 0.5, 0.25, 0.125, 0.063, 0.031 and 0.016 ng/mL solutions. EIA Diluent served as the zero standard (0 ng/mL).
4. Biotinylated Human IFN-ɣ Antibody (100X) was spun down briefly and diluted 1:100 with EIA Diluent.
5. Wash Buffer Concentrate (20X) was diluted to a 1X concentration using distilled water.
6. Streptavidin-Peroxidase (SP) Conjugate (100X) was spun down briefly and diluted 1:100 with EIA Diluent.

Assay procedure

1. 50µL of standard or sample was added per well. After adding sample to the last well, they were covered and incubated for 2 hr.
2. The wells were manually washed five times manually with 200µL of Wash buffer. The plate was inverted and the contents decanted after each wash; then hit 4-5 times on absorbent material to completely remove the liquid.
3. 50µL of Biotinylated Human IFN-ɣ Antibody was added to each well and incubated for 2 hr.
4. The wash procedure was repeated as described above.
5. 50µL of SP Conjugate was added to each well and incubated for 30 min.
6. The wash procedure was repeated as described above.

7. 50µL of Chromogen Substrate was added per well and incubated for about 15 min or till the optimal blue colour density develops. The plate was gently tapped to ensure thorough mixing.
8. 50µL of Stop Solution was added to each well and the colour changed from blue to yellow.
9. Absorbance values were obtained using a microplate reader at a wavelength of 450nm immediately.

APPENDIX 7

Determination of Human TNF-α concentration

This was done using pre-coated Human ELISA kits obtained from Assaypro, USA. Samples were diluted 1:4 in distilled water before use after being brought to room temperature.

Reagent preparation

1. The MIX Diluent Concentrate (10X) was diluted 1:10 with distilled water.
2. The 3ng of Human TNF-α Standard was reconstituted with 3mL of MIX Diluent to generate a solution of 1ng/mL. This was allowed to sit for 10 min and gently agitated before the dilutions were made.
3. The standard solution was diluted serially to produce 0.5, 0.25, 0.125, 0.063, 0.031 and 0.016 ng/mL solutions. MIX Diluent served as the zero standard (0 ng/mL).
4. Biotinylated Human TNF-α Antibody (50X) was spun down briefly using a centrifuge and diluted 1:50 with MIX Diluent.
5. Wash Buffer Concentrate (20X) was diluted to 1X concentration using distilled water.
6. Streptavidin-Peroxidase (SP) Conjugate (100X) was briefly spun down and diluted 1:100 with MIX Diluent.

Assay procedure

1. 50μL of standard or sample was added per well. The wells were covered and incubated for 2 hr after the last sample addition.
2. The wells were washed five times manually with 200μL of Wash buffer. The plate was inverted and the contents decanted after each wash; then hit 4-5 times on absorbent paper material to completely remove the liquid.
3. 50μL of Biotinylated Human TNF-α Antibody was added to each well and incubated for 2 hr.
4. The microplate was washed as described above.
5. 50μL of SP Conjugate was added to each well and incubated for 30 min.
6. The microplate was washed as described above.
7. 50μL of Chromogen Substrate was added per well and incubated for about 15 min or till the optimal blue colour density develops. Gentle tapping of the plate was done to ensure thorough mixing.

8. 50μL of Stop Solution was added to each well and the colour changed from blue to yellow.
9. Absorbance values were obtained using a microplate reader at a wavelength of 450nm immediately.

APPENDIX 8

Determination of Human IL-1α concentration

This was done using pre-coated Human ELISA kits obtained from Assaypro, USA. Samples were used undiluted for optimum readings after being brought to room temperature.

Reagent preparation

1. The EIA Diluent Concentrate (10X) was diluted 1:10 with distilled water.
2. The 250pg of Human IL-1α Standard was reconstituted with 1mL of EIA Diluent to generate a solution of 250pg/mL. This was allowed to sit for 10 min with gentle agitation prior to making dilutions.
3. The standard solution was diluted 1:4 with EIA Diluent serially to produce 62.5, 15.63, 3.906 and 0.977 pg/mL solutions. EIA Diluent served as the zero standard (0 pg/mL).
4. Biotinylated Human IL-1α Antibody (100X) was spun down briefly and diluted 1:100 with EIA Diluent.
5. Wash Buffer Concentrate (20X) was diluted 1:20 with distilled water.
6. Spinning down of Streptavidin-Peroxidase (SP) Conjugate (100X) was done briefly using a centrifuge and then diluted 1:100 with EIA Diluent.

Assay procedure

1. 50µL of standard or sample was added per well. After the last sample addition, the wells were covered and incubated for 2 hr.
2. The wells were washed five times manually with 200µL of Wash buffer. The plate was inverted each time and the contents decanted; then hit 4-5 times on absorbent paper material to completely remove the liquid.
3. 50µL of Biotinylated Human IL-1α Antibody was added per well and incubated for 2 hr.
4. The microplate was washed as described above.
5. 50µL of SP Conjugate was added to each well and incubated for 30 min.
6. The microplate was washed as described above.
7. 50µL of Chromogen Substrate was added per well and incubated for about 20 min or till the optimal blue colour density develops. Gentle tapping of the plate was done to ensure thorough mixing.

8. 50μL of Stop Solution was pipetted to each well and the colour changed from blue to yellow.
9. The absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 9

Determination of Human IL-6 concentration

This was done using pre-coated Human ELISA kits obtained from Assaypro, USA. Samples were diluted 1:4 in distilled water for optimum readings after being brought to room temperature.

Reagent preparation

1. The MIX Diluent Concentrate (10X) was diluted 1:10 with distilled water.
2. Recomposition of 2ng of Human IL-6 Standard was done using 2mL of MIX Diluent to produce 1ng/mL solution. This was allowed to sit for 10 min with gentle shaking of the plate before making dilutions.
3. The standard solution was diluted 1:2 serially to produce 0.5, 0.25, 0.125, 0.063, 0.031, 0.016 and 0.0.008 ng/mL solutions. MIX Diluent served as the zero standard (0 ng/mL).
4. Biotinylated Human IL-1α Antibody (50X) was spun down briefly and diluted 1:50 with MIX Diluent.
5. Wash Buffer Concentrate (20X) was diluted 1:20 with distilled water.
6. Streptavidin-Peroxidase (SP) Conjugate (100X) was spun down briefly and diluted 1:100 with MIX Diluent.

Assay procedure

1. 50µL of standard or sample was added per well. The wells were covered and incubated for 2 hr after adding the last sample.
2. The wells were washed five times manually with 200µL of Wash buffer. The plate was inverted each time and the contents decanted; then hit 4-5 times on absorbent paper material to completely remove the liquid.
3. 50µL of Biotinylated Human IL-6 Antibody was added to each well and incubated for 2 hr.
4. The microplate was washed as described above.
5. 50µL of SP Conjugate was added to each well and incubated for 30 min.
6. The microplate was washed as described above.
7. 50µL of Chromogen Substrate was added per well and incubated for about 12 min or till the optimal blue colour density develops. The plate was gently tapped to ensure thorough mixing.

8. 50μL of Stop Solution was added per well and the colour changed from blue to yellow.
9. Absorbance values were obtained using a microplate reader at a wavelength of 450nm immediately.

APPENDIX 10

Determination of Human IL-10 concentration

This was done using pre-coated Human ELISA kits obtained from Assaypro, USA. Samples were diluted 1:2 in distilled water for optimum readings after being brought to room temperature.

Reagent preparation

1. The MIX Diluent Concentrate (10X) was diluted 1:10 with distilled water.
2. The 16ng of Human IL-1α Standard was reconstituted with 2mL of MIX Diluent to generate a standard solution of 8ng/mL. This was allowed to sit for 10 min with gentle shaking of the plate before dilutions were made.
3. The standard solution was diluted 1:2 with equal volume of MIX Diluent serially to produce 4, 2, 1, 0.5, 0.25 and 0.125 ng/mL solutions. MIX Diluent served as the zero standard (0 ng/mL).
4. Biotinylated Human IL-10 Antibody (100X) was spun down briefly and diluted 1:100 with MIX Diluent.
5. Wash Buffer Concentrate (20X) was diluted to a concentration of 1X using distilled water.
6. Streptavidin-Peroxidase (SP) Conjugate (100X) was spun down briefly and diluted 1:100 with MIX Diluent.

Assay procedure

1. 50µL of standard or sample was added per well. The wells were covered and incubated for 2 hr after the last sample addition.
2. Manual washing of the wells was done five times with 200µL of Wash buffer per wash. The plate was inverted each time and the contents decanted; then hit 4-5 times on absorbent paper material to completely remove the liquid.
3. 50µL of Biotinylated Human IL-10 Antibody was added to each well and incubated for 2 hr.
4. Washing procedure was repeated as described above.
5. 50µL of SP Conjugate was added to each well and incubated for 30 min.
6. Washing procedure was repeated as described above.
7. 50µL of Chromogen Substrate was added to each well and incubated for about 15 min or till the optimal blue colour density develops. The plate was gently tapped to ensure thorough mixing.

8. 50µL of Stop Solution was added to each well and the colour changed from blue to yellow.
9. The absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 11

Determination of Human IL-2 concentration

This was done using pre-coated Human ELISA kits obtained from GenWay Biotech Inc. Samples were not diluted.

Reagent preparation

1. The Biotin-Conjugated Detection Antibody was reconstituted in 25µL of distilled water to make a concentration of 83µg/mL.
2. Protein Standard was reconstituted in 83µL of distilled water for a concentration of 1µg/mL.
3. Wash Buffer Concentrate (10X) was diluted 1:10 with distilled water.
4. The standard solution was diluted 1:2 with equal volume of Assay Diluent serially to produce 4000, 2000, 1000, 500, 250, 125 and 62.5 pg/mL solutions. Assay Diluent served as the zero standard (0 pg/mL).

Assay procedure

1. 100µL of standard or sample was added per well. The wells were covered and incubated for 2 hr after the last sample addition.
2. The wells were washed four times manually with 300µL of Wash buffer. The plate was inverted each time and the contents decanted; then hit 3-4 times on absorbent paper material to completely remove the liquid.
3. The detection antibody solution was then diluted 1:400 in detection antibody diluent.
4. 100µL of diluted detection antibody was added to each well and incubated for 2 hr.
5. The microplate was washed as described above.
6. 400x Streptavidin-HRP was diluted 1:400 using Assay Diluent.
7. 100µL of 1x Streptavidin-HRP was added to each well and incubated for 30 min at room temperature.
8. The microplate was washed as described above.
9. 100µL of TMB substrate solution was added per well and monitored until the blue colour has ceased to develop further.
10. 100µL of Stop Solution was added to each well and the colour changed from blue to yellow.

11. The absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 12

Determination of Human IL-4 concentration

This was done using pre-coated Human ELISA kits obtained from GenWay Biotech Inc. Samples were diluted 1:2 in distilled water for optimum readings after being brought to room temperature.

Reagent preparation

1. The 10,000pg/mL standard solution was diluted with 1mL sample diluent buffer. It was then serially diluted to produce 1000, 500, 250, 125, 62.5, 31.2 and 15.6pg/mL. Sample diluent served as the zero standard (0 pg/mL).
2. Biotinylated anti-human IL-4 was diluted 1:100 with antibody diluent buffer and mixed thoroughly.
3. Avidin-Biotin-Peroxidase Complex (ABC) was diluted 1:100 with the ABC dilution buffer and mixed thoroughly.
4. Wash buffer was prepared by diluting 0.01M PBS in 1 litre distilled water.
5. The ABC working solution and TMB colour developing agent were kept warm at 37°C for 30 min before use.

Assay procedure

1. 100µL of standard or sample was added per well. The wells were covered and incubated for 90 min at 37°C.
2. The plate content was discarded and blotted onto paper towels.
3. 100µL of biotinylated anti-human IL-4 antibody working solution was added into each well and incubated for 60 min at 37°C.
4. The wells were washed three times manually with 300µL of Wash buffer. Wash buffer was allowed to stay 1-2 min in the wells then the plate was inverted each time and the contents decanted; then hit 3-4 times on absorbent material to completely remove the liquid.
5. 100µL of prepared ABC working solution was added to each well and incubated for 30 min at 37°C.
6. The wells were washed five times manually with 300µL of Wash buffer. The wash procedure described above was repeated.
7. 90µL of prepared TMB colour developing agent was added per well and incubated in the dark for 25-30 min at 37°C.

8. 100µL of TMB stop solution was added to each well and the colour changed from blue to yellow.
9. The O.D absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 13

Determination of Human IL-12(p70) concentration

This was done using pre-coated Human ELISA kits obtained from GenWay Biotech Inc. Samples were diluted 1:2 in distilled water for optimum readings after being brought to room temperature.

Reagent preparation

1. The 10,000pg/mL standard solution was diluted with 1mL sample diluent buffer. 0.05mL of the solution was added to 0.95mL of sample diluent buffer and mixed thoroughly for a 500pg/mL standard solution.
2. It was then serially diluted to produce 250, 125, 62.5, 31.2, 15.6 and 7.8pg/mL. Sample diluent served as the zero standard (0 pg/mL).
3. Biotinylated anti-human IL-12(p70) was diluted 1:99 with antibody diluent buffer and mixed thoroughly.
4. Avidin-Biotin-Peroxidase Complex (ABC) was diluted 1:99 with the ABC dilution buffer and mixed thoroughly.
5. Wash buffer was prepared by diluting 0.01M PBS in 1 litre distilled water.
6. The ABC working solution and TMB colour developing agent were kept warm at 37°C for 30 min before use.

Assay procedure

1. 100µL of standard or sample was added per well. The wells were covered and incubated for 90 min at 37°C.
2. The plate content was discarded and blotted onto paper towels.
3. 100µL of biotinylated anti-human IL-4 antibody working solution was added into each well and incubated for 60 min at 37°C.
4. Manual washing of the wells was done three times manually with 300µL of Wash buffer. Wash buffer was allowed to stay 1 min in the wells then the plate was inverted each time and the contents decanted; then hit 3-4 times on absorbent material to completely remove the liquid.
5. 100µL of prepared ABC working solution was added to each well and incubated for 30 min at 37°C.
6. The wells were washed five times manually with 300µL of Wash buffer. Wash buffer was allowed to stay in the wells for 1-2 min then the plate was inverted

each time and the contents decanted; then hit 3-4 times on absorbent material to completely remove the liquid.

7. 90µL of prepared TMB colour developing agent was added per well and incubated in the dark for 15-20 min at 37°C.
8. 100µL of TMB stop solution was added to each well and the colour changed from blue to yellow.
9. The O.D absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 14

Determination of Human IL-13 concentration

This was done using pre-coated Human ELISA kits obtained from GenWay Biotech Inc. Samples were not diluted.

Reagent preparation

1. The Biotin-Conjugated Detection Antibody was reconstituted in 25µL of distilled water to make a concentration of 83µg/mL.
2. Protein Standard was reconstituted in 83µL of distilled water for a concentration of 1µg/mL.
3. Wash Buffer Concentrate (10X) was diluted 1:10 with distilled water.
4. The standard solution was diluted 1:2 with equal volume of Assay Diluent serially to produce 8000, 4000, 2000, 1000, 500, 250, 125 and 62.5 pg/mL solutions. Assay Diluent served as the zero standard (0 pg/mL).

Assay procedure

1. 100µL of standard or sample was added per well. The wells were covered and incubated for 2 hr after the last sample addition.
2. The wells were washed four times manually with 300µL of Wash buffer. The plate was gently shaken between each consecutive wash. The plate was inverted each time and the contents decanted; then hit 3-4 times on absorbent material to completely remove the liquid.
3. The detection antibody solution was then diluted 1:400 in detection antibody diluent.
4. 100µL of diluted detection antibody was added to each well and incubated for 2 hr.
5. The microplate was washed as described above.
6. 400x Streptavidin-HRP was diluted 1:400 using Assay Diluent.
7. 100µL of 1x Streptavidin-HRP was added to each well and incubated for 30 min at room temperature.
8. The microplate was washed as described above.
9. 100µL of Ready-to-Use substrate solution was added per well and monitored until the blue colour has ceased to develop further.

10. 100μL of Stop Solution was added to each well and the colour changed from blue to yellow.
11. The absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 15

Determination of Human IL-17 concentration

This was done using pre-coated Human ELISA kits obtained from GenWay Biotech Inc. Samples were diluted 1:2 in distilled water for optimum readings after being brought to room temperature.

Reagent preparation

1. The 10,000pg/mL standard solution was diluted with 1mL sample diluent buffer. It was then serially diluted to produce 1000, 500, 250, 125, 62.5, 31.2 and 15.6pg/mL. Sample diluent served as the zero standard (0 pg/mL).
2. Biotinylated anti-human IL-17 was diluted 1:100 with antibody diluent buffer and mixed thoroughly.
3. Avidin-Biotin-Peroxidase Complex (ABC) was diluted 1:100 with the ABC dilution buffer and mixed thoroughly.
4. Wash buffer was prepared by diluting 0.01M PBS in 1 litre distilled water.
5. The ABC working solution and TMB colour developing agent were kept warm at 37°C for 30 min before use.

Assay procedure

1. 100µL of standard or sample was added per well. The wells were covered and incubated for 90 min at 37°C.
2. The plate content was discarded and blotted onto paper towels.
3. 100µL of biotinylated anti-human IL-17 antibody working solution was added into each well and incubated for 60 min at 37°C.
4. The wells were washed three times manually with 300µL of Wash buffer. Wash buffer was allowed to stay 1-2 min in the wells then the plate was inverted each time and the contents decanted; then hit 3-4 times on absorbent material to completely remove the liquid.
5. 100µL of prepared ABC working solution was added to each well and incubated for 30 min at 37°C.
6. The wells were washed five times manually with 300µL of Wash buffer. Wash procedure was carried out as described above.
7. 90µL of prepared TMB colour developing agent was added per well and incubated in the dark for 15-20 min at 37°C.

8. 100µL of TMB stop solution was added to each well and the colour changed from blue to yellow.
9. The O.D absorbance was read on a microplate reader at a wavelength of 450nm immediately.

APPENDIX 16

Serum iron determination

Procedure

1. Samples were diluted 1:2 with a 20% (w/v) trichloroacetic acid solution in a polyethylene tube.
2. The tube was capped loosely, mixed and heated in a heating block at 90°C for 15 min.
3. This was allowed to cool and then centrifuged 3000g for 5 min.
4. The supernatant was removed into a clean polyethylene tube.
5. The level of iron in each sample was determined using Atomic Absorption Spectrophotometer (Perkin-Elmer Corporation, 1996).

APPENDIX 17

Sample ELISA Result

Plate1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.714	0.567	0.455	0.594	0.429	0.624	0.559	0.467	0.379	0.445	0.133	0.393
B	0.643	0.472	0.494	0.559	0.504	0.545	0.485	0.435	0.586	0.441	0.292	0.548
C	0.544	0.481	0.497	0.456	0.506	0.469	0.502	0.462	0.379	0.360	0.304	0.324
D	0.536	0.474	0.486	0.483	0.497	0.509	0.473	0.572	0.366	0.364	0.354	0.371
E	0.505	0.486	0.490	0.496	0.435	0.455	0.490	0.567	0.562	0.355	0.577	0.444
F	0.460	0.535	0.459	0.505	0.446	0.563	0.464	0.501	0.644	0.393	0.336	0.414
G	0.478	0.552	0.463	0.509	0.474	0.603	0.433	0.476	0.436	0.429	0.434	0.463
H	0.471	0.522	0.671	0.487	0.448	0.447	0.471	0.467	0.486	0.397	0.421	0.551

Settings Information
Endpoint
Lm1 450
More Settings
Shake Once
Calibrate On
Column Priority

Read Information
SpectraMax Plus384
ROM v1.23 Jun 19 2008
Start Read : 5:29 PM
12/23/2014

Mean Temperature : 27.6 °C

Reduction Settings
Optical Density
Wavelength Combination : !Lm1

Standards

Sample	Concentration pg/mL	BackCalcConc	Wells	Value	MeanValue	SD	CV
01	4000.000	3930.996	A1	0.714	0.714	0....	0.0
02	2000.000	2206.164	B1	0.643	0.643	0....	0.0
03	1000.000	744.094	C1	0.544	0.544	0....	0.0
04	500.000	648.689	D1	0.536	0.536	0....	0.0
05	250.000	344.318	E1	0.505	0.505	0....	0.0
06	125.000	Range?	F1	0.460	0.460	0....	0.0
07	62.500	116.470	G1	0.478	0.478	0....	0.0

Smallest standard value: 0.460

Largest standard value: 0.714