

Cellular Immune Responses to HIV and *falciparum* Malaria Co-infection among Pregnant Mothers

Adeoti O.M^{1,2,*}, Awobode H.O³, Anumudu C.I²

¹The Polytechnic Ibadan, Saki Campus

²Cellular Parasitology Programme, Department of Zoology, University Ibadan, Nigeria

³Parasitology Unit, Department of Zoology, University of Ibadan, Nigeria

*Corresponding author: txy23m@yahoo.com

Received February 27, 2015; Revised April 11, 2015; Accepted April 15, 2015

Abstract Co-infection of malaria and HIV often result in a precarious switch of the immune reaction from T-helper 1 (Th-1) - type 1 to type 2 or vice versa. Immunological status are seriously threatened in the case of infection by HIV and Plasmodium falciparum or both in pregnant mother, hence the need to investigate the immunological responses at different status of infections. A total of 149 mothers were recruited in a longitudinal study in the endemic area of Saki. Rapid diagnostic tested kits were used for HIV diagnosis in mother; CD4⁺ counts were enumerated by using FACS count techniques. Four cytokines were profiled TNF- α , IL-2, IL-10 and IFN- γ by Enzyme Linked Immunosorbent Assay. The data generated were analyzed using appropriate method. The prevalence of malaria in mothers was 85/149 (57.1%); a higher mean parasite density of 340×10^3 parasite/ μ L of blood was observed in multigravidae than in secundigravidae with 195×10^3 parasite/ μ L of blood and 265×10^3 parasite/ of blood in primigravidae, there was no significant relation between parity and parasite density in the participating mothers. The HIV and malaria was (22.8%) in mothers while the co infection of HIV and sero-prevalence was (30.2%). The CD4⁺ counts below WHO recommended were observed in 46.9, 33.4 and 47.1% HIV positive, malaria positive and co-infection mothers respectively. However, the concentration differentials of TNF- α , IL-10, IL-2 and IFN- γ in sero-positive mothers were -0.025, -0.0102, +0.0357 and 0.0255 pg/ μ L respectively. The CD4- T-lymphocytes counts, predominance of Th2 and proportion of co-infection mothers suggest significant health risk.

Keywords: cytokine, co-infection, plasmodium falciparum, sero-prevalence, parity, profiled

Cite This Article: Adeoti O.M, Awobode H.O, and Anumudu C.I, "Cellular Immune Responses to HIV and *falciparum* Malaria Co-infection among Pregnant Mothers." *American Journal of Biomedical Research*, vol. 3, no. 2 (2015): 13-20. doi: 10.12691/ajbr-3-2-1.

1. Introduction

Sub-Saharan Africa represents the region most heavily affected by both malaria and HIV [1] The overlap of these two infections is common and it would be important to understand their interactions and their correct management in order to limit their clinical burden [2] Today, approximately 50% of the world's population, or 3.3 billion people, are at varying degrees of risk of malaria. During the acute bouts of clinical malaria, plasma HIV RNA level rise [3,4] and CD4 cells decline by approximately 40 cells/ μ L/year with each malaria episodes [5]. The interaction between *falciparum* malaria infection with HIV and the involvement of the cellular immune system in determining disease pathogenesis is still far from being elucidated. Malaria infection is also associated with strong CD4⁺ cell activation and up-regulation of pro-inflammatory cytokines, providing an ideal micro-environment for the spread of the virus among CD4⁺ cells and thus, for rapid HIV replication [6]. Therefore, the need to determine the impact of *P.*

falciparum and HIV co-infection on the number of CD4⁺ cells and levels of cytokines produced in pregnant women. Some early studies found no association between HIV and malaria disease severity in adults [7]. More recent research has shown that HIV infection predispose to more frequent episodes of symptomatic malaria [8] and more episodes of severe malaria including death adult [9].

An inverse relationship was found between incidence of severe malaria and CD4⁺ T lymphocyte counts. It is known that co-infected pregnant women are particularly at risk of complications due to interactions between malaria and HIV; pregnant women are more likely susceptible to malaria disease than non-pregnant women [10]. High levels of parasitaemia and chronic parasite infection in placental blood can lead to consumption of nutritive blood substances, to a worsening of perinatal outcomes and to increased rates of maternal morbidity. Malaria and HIV infections were associated with several negative outcomes in newborns of co-infected mothers. [11] demonstrated an association between co-infection and an increased risk of stillbirth and preterm delivery. The two infections were independently associated with an increased risk of low birth weight and foetal growth retardation compared to

HIV-uninfected women and malaria infected women [11]. A study conducted by [12] in Malawi reported significantly higher mortality rates among children born to HIV-sero-positive compared to HIV seronegative women, and the risk of neonatal mortality was greater in co-infected mothers compared to mono-infected mothers. As previously mentioned, malaria promotes HIV RNA replication: a study showed that women had about 2-fold increase in HIV RNA both in peripheral and placental blood, with a consequent higher possibility of mother-to-child transmission of HIV [3].

P. falciparum infections promote macrophages and CD4⁺ cells to activate viral transcription. A high parasite density elicits a strong immune response, leading to a high turnover of HIV RNA and fever; this then indicate cytokine response that raise HIV RNA concentrations [13]. HIV infection targets the immune system leading to a state of immunodeficiency in a setting of immune activation.

2. Methodology

Saki is a border town in Oyo State Nigeria bounded by Kwara State in the North and to the West by Cotonou (Republic of Benin) and Lome (Togo). It comprises of three Local Government Areas, viz: Saki West, Saki East and ATISBO (Ago-Are, Tede, Irawo, Sabe, Baasi, and Oje-Owode). The selected hospitals are located in Saki in the region with mesoendemic *P. falciparum* infection. According to the 2006 National Census figures, Saki is the third largest city in Oyo state with a population density of 189,700. The town is heterogeneous comprising the main people of the Yoruba ethnic group who speak the Yoruba language and in the minority; other ethnic groups which include the Fulani, Togolese, Beninois, and the Tangitas. The study design was of longitudinal study which lasted for 18-month period (August, 2009- February, 2011).

Subjects included in the study gave informed verbal consent and the study was approved by the Joint Ethical Committee UI/UCH and the concerned hospitals. Other ethical considerations as contained in the National Code of Health Research Ethics [14] were followed. Each respondent was pre- and post-counseled after informed consent was given. The questionnaire was drafted and given to an expert in the Faculty of Education at the University of Ibadan for content and face validities. A pilot test was carried out on fifty pregnant volunteers after necessary corrections had been effected, in order to establish the reliability of the instrument. Structured questionnaires were used to collect baseline demographic data (e.g. age, sex, household size etc), clinical data/history, anthropometric data, and head circumference, Last menstrual date, estimated date of delivery, use of IPT since gestation, number of previous pregnancies (gravidity), gestation, history, income status, educational status, history of previous pregnancies. The target population was pregnant women who reported for routine ante-natal check-up. Pregnant mothers who consented to participate in the study after being adequately informed of the project objectives, protocol and benefits were then enrolled. All subjects were encouraged to deliver their babies at the selected hospitals of the project.

From enrollment until delivery, each woman enrolled was followed-up until few months after delivery. All the

enrollees were subsequently reached through medical staff of each Hospital. At each antenatal and post delivery visit, information on occurrence of symptoms, illness, and treatment taken since the last visit to the project was collected on standardized questionnaires. All women recruited received Insecticide Treated Mosquito Nets (LLINs) and free SP (IPT) according to the WHO recommendation.

2.1. Laboratory Procedures

Blood of (5mL) was collected from each patient, 2mL into sterile EDTA bottles which were taken to the Laboratory and another 2 mL into vacutainer bottles. The vacutainer tubes were immediately transported to the General Hospital for CD4⁺ analysis. The EDTA samples were moved under ice to the Department of Science Laboratory Technology Laboratory. The remaining blood was used for thin and thick blood smear for malaria parasitemia, PCV and filter paper preparation. Within six hours of sample collection CD4⁺ total enumeration was performed on the whole blood transported to General Hospital using BD FACS Count CD4 technique. At SLT Department, the EDTA blood sample was centrifuged at 400 rpm in the Laboratory in order to obtain the plasma which was subsequently used to run the ELISA assay. The plasma samples remained stored at -70°C until analysis. In the babies, cord blood samples were collected at delivery for parasitological studies.

2.2. Microscopy

After cleaning the upper surface of the arm with cotton wool moistened with methylated spirit, peripheral blood samples were collected in sterile containers. Thin and thick blood smears were made from each of these samples. A staining protocol using 30% Giemsa stain for thick smear for 15 minutes and Leishman stain was used for thin smear. Parasite counts standardized per 200 leukocytes were performed on thick/thin blood films. The number of parasites per microliter of blood was calculated by assuming an average white blood cell count of 8,000/ μ L. The preparation of both thick and thin blood films for confirmation of parasitemia followed the methods described [15]. The degree of parasitemia was graded by modification of [16] thus (1-999/ μ L) as mild Low and High above (>1000-9999/ μ L). A drop of immersion oil was placed on the stained thin film and another drop on thick film. They were systematically examined using x 100 objectives (oil immersion). A negative result was recorded after thorough examination of 100 fields without any parasite. Quality control was ensured, by using freshly reconstituted filter Giemsa stains for parasitemia.

2.3. HIV Screening

All participants who consented to participate were screened for the presence of HIV antibody by using Determine HIV rapid test kits and/or UNI-Gold Rapid Test grouped into positive reactive and negative samples according to [17]. All those tested positive to RDT kits were later subjected to a confirmatory test at UCH through the Baptist Medical Centre, Saki. Infection status was determined according to the method of [18].

2.4. Measurement of Cytokines in the Plasma by ELISA

Peripheral plasma concentrations of IL-10, IL-2, IFN- γ and TNF- α were measured in all the samples of 179 participants by using cytokine ELISA techniques. The cytokine detection kits purchased from (Mabtech, Stockholm, Sweden) were used for this assay. ELISA plates (Corning Incorporated, Nunc Maxicorb) were coated with the purified anti-cytokine capture monoclonal antibody to (1 μ g/ml) in a coating buffer (Sodium bicarbonate). Monoclonal antibodies used were TNF- α , I-D1K, 9D7 and IL-2-I for TNF- α , IFN- γ , IL-10 and IL-2 respectively. The average ODs were calculated and its corresponding concentrations on the x-axis of the standard curve. Each final value was presented in the nearest $\times 10^{-3}$ pg/ μ L.

2.5. Flourescent Antibody Count Systems

FACS technique is designed to be used with human whole blood samples. The whole blood samples was collected in vacutainer tubes and stored no longer than 6 hours of room temperature at 20-25 $^{\circ}$ [19].

After the additions of CD4 $^{+}$ and CD3 $^{+}$ monoclonal antibody (10 μ L), the samples were incubated and analyzed; immediately it was read on FACS Count Machine according to the manufacturer's instructions (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and a standard six-tube, two-color monoclonal antibody panel (Becton Dickinson). Controls were prepared by adding normal whole blood, and then fixative solution was added to the CD4 reagents tubes followed by addition of control beads. For this study, absolute CD4 $^{+}$ T lymphocytes counts were used to classify patients into three categories: patients with less than 100 cells/ μ L of blood were classified as seriously immunocompromised, those with between 100-350 cells/ μ L of blood were immunosuppressed, the other category were those whose CD4 counts fall above 350 cells/ μ L of blood were regarded as stable [20,21].

2.6. Statistical Analysis

Subjects were grouped according to infection status: HIV negative, malaria negative (no-infection); HIV positive, malaria negative (HIV only); HIV negative, malaria positive (Malaria only) and HIV positive, malaria positive (co-infection) adapting a similar procedure of [22]. All data collected were analyzed using SPSS version 15 according to the protocol described by [23]. Demographic characteristics at baseline and the variations in the CD4 cell counts in the sample cohort were analyzed. The mean CD4 cell count (and its variance) by co-infected participants were then compared using Students t-tests (for characteristics with two categories) and ANOVA tests. The significance limit was $p=0.05$

3. Results

A total of 149 pregnant women participated in the study. The general descriptions and schematic description of the categories of the study participants were as shown in (Figure 1). Among the participants, several groups were identified based on their infection status. There was the group infected with HIV only (30.2%), and that infected with *P. falciparum* (57.1%). Participants with co-infection of *P. falciparum* and HIV made up another group 39 (21.8 %). (Figure 1). Majority (70%) of the participating pregnant women were in their second trimester and 69 (46.3%) were multigravidae. All the participating women were further stratified based on their gravidity and their *P. falciparum* and HIV infections status. The highest prevalence of *P. falciparum* infection (21.5%) was observed among multigravidae. Malaria infection was found in all gravid states of pregnancy, but seems to be more in the secungravidae. The highest prevalence of HIV infection was observed among the primigravidae than both the secundigravidae and multigravidae and there was a statistically significant association between parity and prevalence of HIV infection.

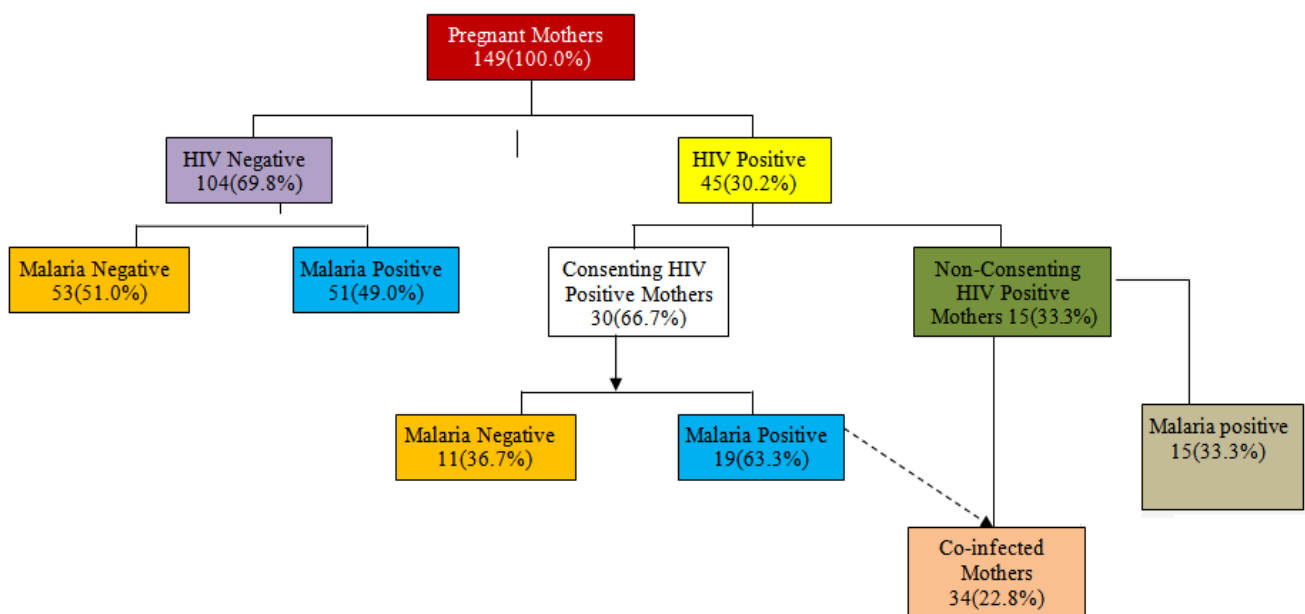


Figure 1. schematic representation of the study

Table 1. Medical Characteristics of Participating Mothers by Gravidity

Characteristics	Primigravidae (%)	Secungravidae (%)	Multigravidae (%)
	G ₁ n=39(32.9)	G ₂ n=41(27.5)	G ₃ 69(39.6)
Parasitemia			
Low	25(30.9)	24(29.6)	32(39.5)
High	16(23.5)	15(22.1)	37(54.4)
Total	41(27.5)	39(26.2)	69(46.3)
RVS status			
Positive	32(34.0)	27(28.7)	37(54.7)
Negative	15(28.3)	14(26.4)	24(45.3)
Total	47(32.0)	41(27.9)	61(40.9)
CD4+ (cells/μL)			
<350*	19(33.9)	11(19.6)	29(49.2)
$\geq 350^{\text{s. (b)}}$ 1532	28(32.6)	27(31.4)	35(36.0)
Total	47(33.1)	38(26.8)	59(41.0)

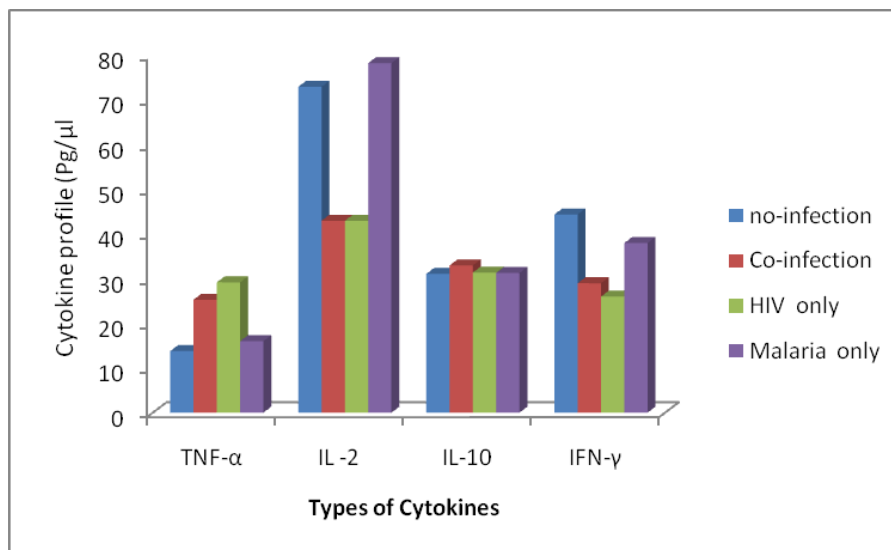
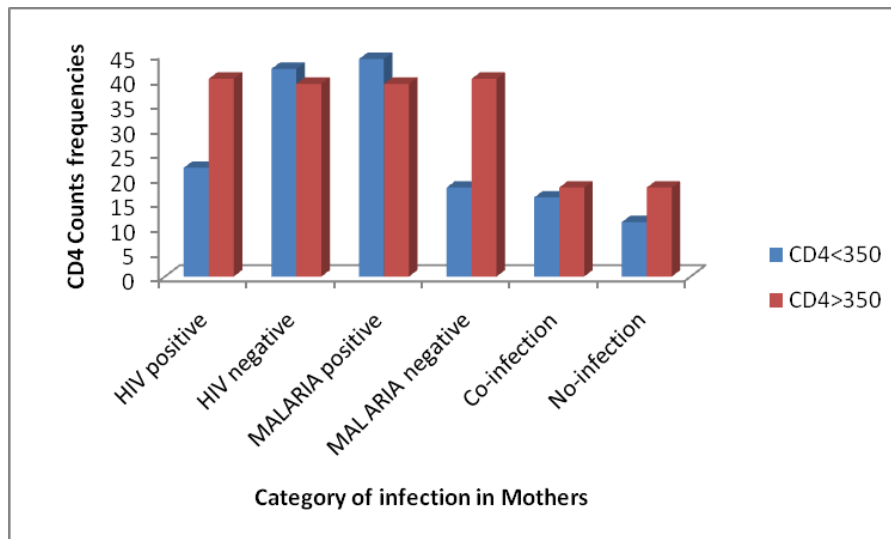


Figure 2. Mean plasma cytokine concentration and the infection status of participants

Figure 3. Infection categories and frequency of CD4⁺ lymphocytes counts among the women participants

4. Haematological parameters among co-infected participants

The mean Hb level of *P. falciparum* and HIV co-infected babies was 8.73 ± 3.13 g/dl and that of the pregnant mothers was 7.49 ± 3.34 g/dl. There was no

significant difference between the means of CD4⁺, haemoglobin level and *P. falciparum* density in women ($p=0.335$) (Table 2). There was a significant difference between the means of CD4⁺, haemoglobin levels and *P. falciparum* density in babies ($p=0.005$). The mean CD4⁺T cell count for the co-infected group was 195 ± 23 cells/ μ l and that of the babies was 220 ± 140 cells/ μ l. However, the mean parasite density in the HIV and *P. falciparum*

co-infected pregnant women was $32,362 \pm 3.43$ parasites/ μL and that of the babies was $4,863 \pm 5.23$ parasites/ μL (Table 2). The CD4^+ lymphocyte count in all respondents were grouped based on categories of infection; the mean CD4 cell counts in all respondents was classified and then compared with the recommended standard

(WHO, 2004). The percentage of CD4^+ counts in HIV infected, *P. falciparum* infected and the *P. falciparum* and HIV co-infected were 46.9% (Figure 3), 53.4% and 47.1% respectively; pregnant women with less than 350 cells/ μL below the WHO standard.

Table 2. Relationship between mean haemoglobin levels, CD4^+ T cell count and parasite density in HIV and malaria co-infected mothers and co-infected babies

Group	No examined	Mean CD4^+		Mean parasite density	
		T-Cells/ $\mu\text{L} \pm \text{SD}$	g/dl	<i>P. falciparum</i>	p-values
Mothers	34	195 ± 23	7.49 ± 3.34	$32,362 \pm 3.43$	0.335**
Babies	05	220 ± 140	8.73 ± 3.13	$4,863 \pm 5.23$	0.005*
Total	39				

**not significant association

*Significant association, Mann-Whitney: $U = 0.580$.

4.1. Cytokine Profiles and Infection Status

In Figure 2, higher mean plasma concentration of $\text{TNF-}\alpha$ was observed from $18.0 \times 10^{-3} \text{pg}/\mu\text{L}$ in un-infected to $28.2 \times 10^{-3} \text{pg}/\mu\text{L}$ in co-infected and $32.3 \times 10^{-3} \text{pg}/\mu\text{L}$ in mothers infected with HIV only, the levels of $\text{IFN-}\gamma$ was low from $50.0 \times 10^{-3} \text{pg}/\mu\text{L}$ in un-infected to $32.0 \times 10^{-3} \text{pg}/\mu\text{L}$ and $28.3 \times 10^{-3} \text{pg}/\mu\text{L}$ in both co-infected and those infected with HIV only. However, low level of $\text{TNF-}\alpha$ was

observed from $30.0 \times 10^{-3} \text{pg}/\mu\text{L}$ in un-infected to $17.0 \times 10^{-3} \text{pg}/\mu\text{L}$, $20.0 \times 10^{-3} \text{pg}/\mu\text{L}$ and $0.8 \times 10^{-3} \text{pg}/\mu\text{L}$ in co-infected HIV only and those infected with *P. falciparum* only. The IL-2 levels reduced from ($78.0 \times 10^{-3} \text{pg}/\mu\text{L}$ to $45 \times 10^{-3} \text{pg}/\mu\text{L}$) in both co-infected and HIV infected mothers then high ($82.0 \times 10^{-3} \text{pg}/\mu\text{L}$) in malaria infected mothers. Also, there were strong significant associations between the plasma IL-2 levels and infections status among the study participants (Table 3).

Table 3. Cytokines levels and infection status of pregnant women

	HIV only	Malaria Only	Co-infection	No-infection	ANOVA
	(n=20) ($\bar{x} \pm S$)	(n=59) ($\bar{x} \pm S$)	(n=25) ($\bar{x} \pm S$)	(n=45) ($\bar{x} \pm S$)	
$\text{TNF-}\alpha$	0.49 ± 0.11	0.39 ± 1.59	0.45 ± 0.12	0.39 ± 0.16	0.02
IL-2	0.26 ± 0.13	0.22 ± 0.22	0.26 ± 0.33	0.22 ± 0.33	0.00
IL-10	0.26 ± 0.53	0.23 ± 0.36	0.26 ± 0.60	0.23 ± 0.34	0.00
$\text{IFN-}\gamma$	0.45 ± 0.08	0.41 ± 0.14	0.48 ± 0.06	0.44 ± 0.12	0.34

In pregnant mothers, the plasma level of IL-10 in the co-infected women was low when compared to the un-infected and those infected with *P. falciparum* only. The concentration of IL-10 in co-infected mothers was slightly lower ($10.0 \times 10^{-3} \text{pg}/\mu\text{L}$) than ($25.3 \times 10^{-3} \text{pg}/\mu\text{L}$). There was no significant relationship between the plasma concentration of IL-10 and HIV status (Table 3). The concentration of $\text{IFN-}\gamma$ in was highest in un-infected ($48.0 \times 10^{-3} \text{pg}/\mu\text{L}$). Also, the concentration of $\text{IFN-}\gamma$ co-infected women ($22.2 \times 10^{-3} \text{pg}/\mu\text{L}$) (Figure 3) was higher than in the un-infected ($22.0 \times 10^{-3} \text{pg}/\mu\text{L}$), lowest in HIV infected ($0.8 \times 10^{-3} \text{pg}/\mu\text{L}$). In general, there was significant association between the plasma levels of $\text{TNF-}\alpha$, IL-2 and $\text{IFN-}\gamma$ and the HIV status of the pregnant women. There was significant association between the plasma concentration of $\text{IFN-}\gamma$ and HIV status (Table 3) and the infection status.

5. Discussion

Both malaria and HIV infection during pregnancy increase the susceptibility of pregnant women to the negative effects of malaria, and also makes them more vulnerable to infections regardless of parity and impaired immune state [23]. The mechanism governing this increased susceptibility of pregnant women to *falciparum* malaria is not well understood [25]. It is presumed to be related to probable serious modifications of both the systemic and immunological placental parameters.

In this study, there was a high prevalence of HIV and malaria co-infection, both infections have been shown to be associated with serious immune disturbance [26] among pregnant mothers. The finding in this study that malaria infection was higher in HIV positive pregnant women than in the non HIV positive women was supported by earlier reports [27]. The low mean birth weight observed in this study is in agreement with studies that predicted poor perinatal outcomes such as low birth weight as consequences of co-infection of HIV and *Plasmodium falciparum* in pregnant women [28]

The underlying cellular immune disruptions that make all parities equally susceptible to infections have not been elucidated. Even though opinions were divided on the true immune state of pregnant mothers as to guarantee successful pregnancy, previous papers [38] hypothesized on the mechanism responsible for cellular consequences of the co-infections during pregnancy and that the increased susceptibility of co-infected individuals is due to excessive destruction of the IL-12 -mediated $\text{IFN-}\gamma$ pathway due to HIV infection [25].

Co-infected pregnant mothers had lower level of haemoglobin which was an indication of anaemia than women with single infection of malaria or HIV (Table 2). Clearly, increased parasitemia can lead to greater destruction of red blood cells [29], dramatic decrease in CD4^+ T cells which can also lead to early progression of HIV to AIDS [30]. The observed relationship between mean haemoglobin level and the CD4^+ cell reported among the co-infected participants was similar to [30] who reported a statistically significant association between

CD4⁺ and haematological parameters. Co-infected women are more likely to be immunosuppressed with lower CD4⁺ especially during the second and third trimester. HIV and malaria co-infected multigravid women showed better improvement towards the treatment than the primigravid ones, although further studies are required to investigate the findings by [31]. Several factors which include nutrition, age, time of sample collection had been documented as factors that could be responsible for the reduced CD4 counts among the un-infected groups [32,33]. Both malaria and HIV cause immunodeficiency by depletion, lysis and infection of CD4⁺ T-lymphocytes [34]. [32] obtained a similar result that led to the geometric increase in parasite density in patients with CD4 count <200/ μ L and 50% higher at CD4 count 200-499/ μ L than uninfected participants.

The high in TNF- α level among those infected with HIV and the co-infected women compared to their uninfected peers (Figure 2), contradict the recent data [36] Ordinarily high concentrations of TNF- α as reported by [37] was an indication of increased HIV-1 expression in the materno-foetal milieu and facilitation of MTCT of HIV-1. Also, [38] indicated that increase in TNF- α cytokine was deleterious to both the mothers and her baby post-partum [38,39].

The reduction in the plasma levels of IL-2 in co-infected and HIV infected when compared to uninfected pregnant women contradicted the work of earlier authors [40,41] on the high concentration of IL-2 in their subjects. Also, the observed high IL-2 levels agree with similar study conducted by [42], where high levels of IL-2 was observed among malaria infected cohort (Figure 2). The protective role of IL-2 as being indispensable in *P. falciparum* has been extensively documented by [36].

IL-10; an anti-inflammatory cytokine inhibits Th-1 stimulating cytokine secretion that will ensure successful pregnancy [39]. The cytokine is thought to play a key role in the regulation of biological parameters in pregnant mothers. Since, no case of spontaneous abortion was reported among groups, it is expected that its level will predominate in this study. Its continuous production could also be associated with *falciparum* placenta infection and anaemia in newborn but on the contrary, the level of IL-10 was reduced among the pregnant women.

The pattern observed in the level of IFN- γ in this study contradicts studies of [42,43]; the concentration of IFN- γ was low upon infection. Low circulating levels of IFN- γ have been previously associated with severe malaria and mortality in children especially infants with cerebral malaria. The concentration of IFN- γ followed the same trend that was observed in the levels of IL-2 in this study. IFN- γ was expected to have played a crucial role in the clearance of intracellular parasites, and associates with high in malaria severity in young African children [44]. Its reduction in all infectious cases (especially in co-infected and HIV infected only) in this study could be regarded as being pathological (biomarker) of possible impaired resistance against infection which confirmed the result obtained in similar study by [45].

[46] established that in newborns of HIV infected mothers, HIV and *falciparum* co-infection was a strong lead predictor of adverse perinatal outcomes (anaemia), and could be indicative of impaired immunity and susceptibility of newborn to infections. The general

reduction in TNF- α level that was observed in this study was a confirmation of that assertion. As suggested earlier, the similar low level observed in newborns of HIV infected could be an indication of vertical transmission of impaired immunity from their HIV infected mothers. This might have led to increased IFN- γ concentration among co-infected babies and malaria only infected. The high level of IFN- γ in non-infected mothers compared to the levels of IL-10 (2) was supposed to be deleterious to successful pregnancy and a comparative indication of severe perinatal outcome and high parasite density which would be potentially harmful for the pregnancy [44,47].

There was no statistical association between CD4⁺ lymphocytes, and IFN- γ levels and HIV infection status of pregnant mothers, and supports the work of [39,47,] who reported that successful pregnancy is dependent of the disequilibrium between Th 1 and Th 2 cells. Also in the study, only IFN- γ was significantly associated with the infection status of the women, this was consistent with the work of [36] The appropriate immune reaction associated with protection in pregnancy is believed to be provided by Th2 cells which inhibit the development of Th1 cells, and hence prevent macrophage from mounting massive immune reactions against pregnancy.

The current study was characterized by low levels of IL-10; this contradicts [48] who associated high levels of IL-10 and low levels of the other cytokines IFN- γ , IL-2 and TNF- α with severe infection and poor pregnancy outcome. Whether protective or not, the levels of IFN- γ , TNF- α and IL-10 in new born of HIV infected mothers should be applied with caution because the switch (Th-1 to Th-2) might not entirely explain HIV progression. Early stage HIV infection as a Th1-predominant profile (characterized by a high production of IL-2 and IFN- γ , while the late HIV infection as a Th-2 predominant profile characterized by IL-4 and IL-10 production. There has been a strong association between levels of pro-inflammatory cytokines and spontaneous abortion [49,50].

References

- [1] Imani, P.D., Musoke, P., Byarugaba, J., Tumwine, J.K.. "Human immunodeficiency virus infection and cerebral malaria in children in Uganda: a case-control study". *Biomedical Central Pediatrics* 11.5: 1471-2431 (2011).
- [2] Idemyor, V., "Human Immunodeficiency Virus (HIV) and malaria interaction in sub-Saharan Africa: the collision of two titans" . *HIV Clinical Trials*. 8:246-253(2007).
- [3] Mwapasa, V., Rogerson, S.J., Molyneux, M.E. "The effect of *Plasmodium falciparum* malaria on peripheral placental HIV-1 RNA concentrations in pregnant Malawian women" *AIDS*, 18:1051-1059 (2004).
- [4] Karp, C.L., Auwaerter, P.G. "Co-infection with HIV and tropical infectious diseases". *Protozoal pathogens Clinical Infectious Disease* 45:1208-13 (2007).
- [5] Mermin, J., Lule, J.R., Ekwaru, J.P. "Association between malaria and CD4 cell count decline among persons with HIV". *Journal of Acquired Immune Deficiency Syndrome*. 41.1: 129-130 (2006).
- [6] World Health Organization. "Malaria and HIV interactions and their implication for public health Policy" report of a WHO technical consultation, Geneva, Switzerland, 23-25 (2004).
- [7] Chandramohan, D. and Greenwood, B.M.. "Is there an interaction between human immunodeficiency virus and *Plasmodium falciparum*?" *International Journal of Epidemiology*. 27. 2: 296-301 (1998).
- [8] Kanya, M.R., Gasasira, A.F., Yeka, A. "Effect of HIV-1 infection on anti-malarial treatment outcomes in Uganda: a population-based study". *Journal of Infectious Diseases*. 193.1: 9-15 (2006).

- [9] Grimwade, K., French, N., Mbatha, D.D., Zungu, D.D., Dedicoat, M., and Gilks, C.F. "Childhood malaria in a region of unstable transmission and high Human Immunodeficiency Virus prevalence". *Pediatric Infectious Disease Journal*. 22.12: 1057-1063 (2003).
- [10] Uneke, C.J. Assessment of malaria in pregnancy using Rapid Diagnostic Tests and its association with *Plasmodium falciparum* parasitaemia. *AIDS*, 13:1145-1146. (2007).
- [11] Ticconi, C., Mapfumo, M., Dorrucchi, M., Naha, N., Tarira, E., Pietropolli, A "Effect of maternal HIV and placental HIV-1 RNA concentrations in pregnant Malawian women" *AIDS*. 18:1051-1059. (2003).
- [12] Whitworth, J., Morgan, D., Quigley, M., Smith, A., Mayanga, B., Eotu, H., Omoding, N., Okongo, M., Malomba, S., Ojwira, A. "Effect of HIV-1 and increasing immunosuppression on malaria parasitemia and clinical episodes in adults in rural Uganda: a cohort study". *Lancet* 356:1051-1056 (2002).
- [13] Lawn, S.D., Butera, S.T., Folks, T.M. "Contribution of immune activation to the pathogenesis and levels determine marked differences in Mother-To-Child Transmission rates of HIV- 1 and HIV-2 in the Gambia". *AIDS*. 14:441-448. (2001).
- [14] Federal Ministry of Health. National code of health research ethics ISBN: 978-978-080-708-5 cited at <http://www.nhrec.net> (2007).
- [15] Cheesbrough, M. District Laboratory practice in Tropical countries. 2nd edition Cambridge: *Cambridge University Press* 244-51 (2005).
- [16] Ogbodo, S.O., Nwagha, U.I., Okaka, O.N.C., Ogenyi, S.C., Okoko, R.O., Nwagha, T.U. "Malaria parasitemia among pregnant women in rural community of eastern Nigeria: need for combined measures". *Nigerian Journal of Physiological Sciences* 24. 2:95-100 (2009).
- [17] World Health Organization. Malaria, a Global Public Health problem. World Malaria Report 2004. Accessed on June 10, 2009 at www.wpro.who.int/sites/mvp/data (2005).
- [18] Amuta, E.U., Ikpa, T.F. "High prevalence of HIV infection, increased tendency to malaria burden and Development of AIDS at the Federal medical centre, Makurdi, Nigeria". *Nigerian Journal of Parasitology* 26: 7-11 (2005).
- [19] Center for Disease Control Department Human Services "Determine HIV rapid test for use with whole blood, serum or plasma women receiving anti-folates in a high transmission area of Uganda". *Malaria Journal* (2006).
- [20] Agbede, O.O., Ajiboye, T.O., Kolawole, O.M., Babatunde, S.A., Odeigha, L.O. Evaluation of CD4⁺ T cells in HIV patients presenting with malaria at University of Ilorin Teaching Hospital Nigeria *EXCLI Journal*, 9:58-66 (2009).
- [21] Tanjong, E., Atashili Kanga J., Ikomey H.I.F., Akenji, G., Nduembe, M.P. "Reference values of CD4⁺ lymphocyte counts in HIV sero-negative pregnant women in Buea, Cameroon". *African Journal of clinical and Experimental Microbiology*. 13. 1:55-69X (2012).
- [22] Abaniyiye, F.A., McCracken, C., Kirrivan, P., Mollo, S.F., Asaolu, S.O., Holland, C.V., Gutman, J., Lamb, J.J. *Ascaris* co-infection does not alter malaria-induced anaemia in a cohort of Nigeria Pre School Children. *BMC Malaria Journal*. 12:1-8 (2013).
- [23] Mayanga, V.P., Minzi, O., Ngasala, B. Prevalence of malaria and anaemia among HIV infected pregnant women receiving cotrimoxazole prophylaxis in Tanzania: a cross sectional study in Kinodomi Mumapahty. *Biomedical Central: Pharmacology and Toxicology*. 15:24 (2014).
- [24] Perrault, S.D., Hajek, J., Zhong, K., Owino, S.O., Sichangi, M., Smith, Geoffrey Smith, Shi, P.Y., Moore, J.M., Kain, C. "Human Immunodeficiency Virus Co-infection increases placental parasite density and transplacental malaria transmission in Western Kenya". *American Journal of Tropical Medicine and Hygiene* 80.1:119-125 (2009).
- [25] Moore, J.M., Ayisi, J., Nahlen, B.L., Misore, A., Lai, A.A., Udhayakumar, V., "Immunity to Placental malaria. II. Placental antigen-specific cytokine responses are impaired in human immunodeficiency virus-infected women". *Concise communication*. 1-2 (2000).
- [26] Ballan, W.M., Bien-aimce, N.V.U., Long, B.R., Loo, C.P., Michealsson, J, Barbour, J.D., Lanjar, L.L., Wiznia, A.A., Abadi, J, Fennelly, G.J., Roserberg M. G., Nixon, D.F., Natural killer cells in perinatally HIV-1 infected children exhibit less degranulation compared to HIV-1 exposed uninfected children and their expression of KIR2DL3, NK72C and NKP46 correlates with disease severity. *Journal of Immunology*. 179: 3362-3370. (2007).
- [27] Adefoye, O.A., Adeyeba, O.A., Hassan, W.O., and Oyeniran, O.A. "Prevalence of malaria parasite infection among pregnant women in Osogbo, Southwest Nigeria". *American and European Journal of Science Research* 2: 43-45 (2007).
- [28] Chalwe, V., Van geertruyden, J., Mukwamataba, D., Menten, J., Kamalamba, J, Mulenga, M. "Increased risk for severe malaria in HIV-1 infected adults, Zambia". *Emerging Infectious Diseases* 15. 5:749-858 (2009).
- [29] Ayouba, A., Badaut, C., Kfutwah, A. Specific stimulation of HIV-1 replication in human placental trophoblasts by an antigen of *Plasmodium falciparum* *AIDS* 22. 6: 785-787 (2008).
- [30] Aryee, D.N., Boachie, J. Assessment of the impact of malaria on CD4⁺ T cells and haemoglobin levels of HIV-malaria co-infected patients. *Journal of Infection and Development*. 6. 9:660-663 (2012).
- [31] van Eijk A.M., Ayisi J.G., ter Kuile F.O. "HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya". *AIDS*. 17.4: 595-60. (2003).
- [32] Van geertruyden, J.P., Menten J., Colebunders, R., Korenromp, E., D'Alessandro, U. "The impact of HIV-1 on the malaria parasite biomass in adults in sub-Saharan Africa contributes to the emergence of anti-malarial drug resistance". *Malaria Journal BioMedCentral*. 7:134 (2012).
- [33] Kimbi, H.K., Njoh, D.T., Ndamukong, K., Lehman, L.G. "Malaria in HIV/AIDS Patients at different CD4⁺ T cell levels in Limbe Cameroon". *Journal of Bacteriology and Parasitology* 4172:2155-97 (2013).
- [34] Nwokedi, E.E., Ochicha, O., Muhammed, A.Z., Saddiq, N.M. . "Baseline CD4 lymphocyte count among HIV patients in Kano, Northern Nigeria". *African Journal of Health Sciences*. 14. 3-4: 210-215 (2007).
- [35] Vangeertruyden J.P. "Interactions between Malaria and Human Immunodeficiency virus". *A Review of Clinical Microbiology*. 10.1111: 1469-1478 (2014).
- [36] Tian, L., Wang, T., Lv, S., Wang, F., Guo, J., Yin, X., Cai, Y., Dickey, M.K., Steinmann, P., and Chen, J. "HIV and intestinal parasite co-infections among a Chinese population: an immunological profile". *Infectious Diseases of Poverty*. 2:18. (2013).
- [37] Kfutwah, A.K.W., Marry, J.Y., Nicola, M.A., Bliase-Boisseau, S., Barre-Sinoussi, F., Ayouba, A., Menu, E., Tumor Necrosis Factor- α stimulate HIV-1 replication in single-cycle infection of Human term placental villi fragments in a time, viral dose and envelope dependent manner. *Retrovirology* 3:36 (2006).
- [38] Singh, N., John, R. Immune Reconstitution Syndrome and Exacerbation of Infections after Pregnancy. *Clinical Infectious Diseases*. 45:1192-1199. (2001).
- [39] Raghuparty, R., Al-Mutawa, E., Makhseed, M., Azizieh, F., Szekeres-Bartho, J. Modulation of cytokine production by dydrogestrone in lymphocytes from women with recurrent miscarriage. *BJOG: an International Journal of Obstetrics and Gynecology*. 112: 1096-1101 (2009).
- [40] Ned, R.M., Moore, J.M., Chaisavaneeyakorn, S., Udhayakumar, V., Modulation of Immune responses during HIV-malaria co-infection in pregnancy. *Trends in Parasitology*. 21.6:284-291 (2005).
- [41] Kfutwah, A., Marry, J.Y., Lemen, B., Leke, R., Rousset, D., Barre-Sinoussi, F., Nerriement, E., Menu, E., Ayouba, A., *Plasmodium falciparum* infection significantly impairs placental cytokine in HIV-Infected Cameroonian women. *PLoS ONE*. 4: 8114-18 (2009).
- [42] Fried, M., Richard, O., Muga, Ambrose, O., Misore, Patrick, E. D. Malaria Elicits Type 1 Cytokines in the Human Placenta: IFN- γ and TNF- α Associated with Pregnancy Outcomes, *Journal of Immunology* downloaded on 5th May, 2012 online at <http://www.jimmunol.org/subscription> (2012).
- [43] Hochman, S., and Kim, K., "The impact of HIV co-infection on cerebral malaria pathogenesis". *American Journal of Tropical Diseases and Hygiene* 71:41-54. (2012).
- [44] Niikura, M., Kamiya, S., Kita, K., and Kobayashi, F. Co-infection with on lethal murine malaria parasites suppresses pathogenesis caused by *Plasmodium berghei* NK65 *Journal of Immunology*. 180.10: 6877-6884. (2012).
- [45] Angulo, I., Fresno, M., Cytokines in the pathogenesis of and protection against malaria. *Clinical and Diagnostic Laboratory Immunology*. 9.6: 1145-1152 (2002).

- [46] Laar, A.K., Grant, F.E., Addo, Y., Soyori, I., Nkansah, B., Abugri, J., Laar, A.S., Ampofo, W.K., Tuakli, J.M., Quakyi, I.A. 2013. Predictors of fetal anemia and cord blood malaria parasitemia among Newborns of HIV-positive mothers. *British Medical Central Research Notes*. 6:350-356 (2000).
- [47] Clerici, M., Seresella, M., Colombo, F., Fossati, S., Sala, N., Bricalli, O., Villa, M.L., Feerrante, P., Dally, L., Vigono, A. "T-Lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV". *BLOOD* 96.12: 3866-3871 (2000).
- [48] Lokossou, A.J., Dechavanne C., Bouraima A., Courtin D., Le Port A., Ladekpo R., Noukpo J., Bonou D., Ahouangninou C., Sabbagh A., Fayomi B., Massougbdji A., Andre G., Garcia A., Migot-Nabias F.. "Association of IL-4 and IL-10 maternal halotypes with immune responses to *P. falciparum* in Mothers and Newborns". *BioMedCentral Infectious Diseases* 13:215-223 (2013).
- [49] El-Far, M., El-Sayed, I.H., El-Motwally, A.E., Hashem, I.A., Bakry, N. "Serum levels of TNF-alpha and antioxidant enzymes and placental TNF alpha expression in unexplained recurrent spontaneous miscarriage". *Journal of Physiology and Biochemistry* 65.2:175-181. (2009).
- [50] Mbugi, V.E., Mejjierink, M., Veene Mans J., Jeurink, P.V., McCall, M., Olomi, R.M., Shao, F.J., and Verhoeff, H., Savelkoul, H.F.J. "Alterations in early cytokine mediated immune responses to *Plasmodium falciparum* infection in Tanzanian children with mineral element deficiencies: a cross-sectional survey". *Malaria Journal*. 9:130 (2010).