Cytokine Profiles Among HIV and Malaria Co-Infected Pregnant Mothers and their Babies Post Delivery

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***Abstract*—M**alaria and HIV co-infected mothers have an increased risk of poor birth outcome, spontaneous abortion, malaria infection and early progression of HIV to AIDS. This study examines the impact of HIV and malaria on the levels of cytokines in peripheral blood from mothers and their new borns. 149 pregnant mothers and 30 babies born to 63 HIV infected mothers were engaged in longitudinal study for 18 months in the endemic area of Saki and Ibadan. Blood samples collected from mothers and babies' cord blood were tested for HIV and malaria parasite. ELISA was used to determine the plasma concentration of TNF-α, IL-2, IL-10 and IFN-γ and statistical tests were considered significant at $P< 0.05$. There was no correlation between HIV status and the profiles of anti-inflammatory cytokines but a direct relationship exists between cytokine levels and malaria infection. The increased levels of IL-10 in HIV-infected mothers could worsen the incidence of falciparum malaria.

***Keywords*—**Cytokines, malaria and HIV, co-infection.

I. Introduction

MALARIA and HIV are the major priority medical challenges in sub-Saharan Africa, and little is known of the immunological implication of their co-infections [24]. Th1-Th-2 cytokines are of particular interest since they are the fundamental messengers of adaptive immunity and, as such are likely to be involved in pregnancy, HIV and malaria infection. Pro-inflammatory (Th1) cytokines such as TNF-α is thought to play a crucial role in successful pregnancy, successful birth outcomes, malaria pathogenesis especially cerebral malaria, and control and pathogenesis of HIV infection [2, 16]. Clinical evidence indicates that pregnant women undergo immunological exchanges consistent with weakening of Th1 and strengthening of Th2 responses leading to the establishment of successful pregnancy [31]. Conversely, the activation of some forms of maternal cellular immunity is potentially hazardous for foetal development. Cellular immunity mediated by effectors cells and/or cytokines released by them have shown significant deleterious effects on the foetus. The injection of TNF-α, IFN-γ and IL-2 into pregnant mice causes abortions while injections of anti-TNF antibodies results in a reduction rates in a murine model of natural, immunologically-mediated abortion [26, 27].

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II. Materials and Methods

A. Study area

Saki is a border town, 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. The selected Hospitals are located in region mesoendemic to P. falciparum infection a typical of what is generally obtainable in sub-Saharan Africa, where quality health facilities are virtually non-existent.

B. Climate and Vegetation

Saki is located in 8o 26' and 9o5' north of the equator and longitudes 2o 45' and 3o 37' East of the meridian. This location confers on the town the equatorial climatic conditions. There are two distinct seasons namely wet and dry seasons. The wet season is the period for rainfall, which is between mid-April and October characterized by double maxima distribution in the Southern part, as a result of Western monsoon wind on the atmosphere (Falling Rain Genomics, 2010). The dry season covers between November and March and it is characterized by hot weather. The rainfall pattern is remarkably constant ranging between 1,211 mm at Kisi (East of Saki). The mean temperature is 33oC while the sunshine hours per day range from 3.4 hours in August to 11 hours in February. Rice and roots crops are the main agricultural products. Indigo is also grown in the surrounding area, making Saki an important center for distribution of dyed products.

C. Population

According to the 2006 population census, Saki was estimated to have a population of 189,700. Its heterogeneous nature could have accounted for its reported high incidence of co-infection of malaria and HIV/AIDS in 2005 [11]

D.The People

The town is heterogeneous, comprising mainly of the Yoruba; other ethnic groups found in the minority include the Fulani, Togolese, Beninois, and the Tangitas. The latter like the Yoruba, claim descent from Oduduwa.

E. Study Design

The study was a longitudinal study covering 18 months period in five contiguous sites in Saki, south-west; Nigeria and University Teaching Hospital, Ibadan. The selected hospitals in Saki were: Baptist Medical Centre, General Hospital, Tunmise maternity Clinic, and Isale-Taba Maternity and Muslim Hospital. At enrolment, after informed consent was sought from the mothers, cord blood samples was collected at delivery few minutes after placental expulsion and 2µL of post-delivery venous blood was collected from each baby, the blood was dispensed into sterile EDTA and vacutainer tubes bottles The remaining 1 µL of blood was used to prepare both thin and thick blood smear for malaria parasitemia, PCV and a drop on Whatmann filter paper. Within six hours of collection CD4+ total enumeration was performed on all the vacutainer blood using FACS count technique while EDTA blood sample was centrifuged at 400 rpm in the Laboratory in order to separate the plasma. The plasma samples were transported under insulated sample box under ice for storage at -70oC in a freezer. The results of all samples collected at delivery were later correlated with all the subsequent follow-up samples. All babies born to HIV-infected whose mothers were enrolled for the study and babies whose mothers declined consent or who were not willing to comply with the follow-up requirements were excluded. All babies enrolled followed-up monthly until six months after delivery. At each postpartum visit, information on occurrence of symptoms, illness, and treatment taken since the last visit to the project was collected on standardized questionnaires and structured interview session.

F. Blood smears for Plasmodium falciparum

A rapid 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 was obtained from thick/thin blood films. The number of parasites per microliter of blood was calculated by assuming an average white blood cell count of 10,000/µL.

The degree of parasitaemia was graded thus: (1-999/µL) as mild +, (1000-9999/µL) as moderate or ++, and (>10000/µL) as severe or +++ according to technique described by [6] . All infant blood samples whose mothers gave consent to participate were transported to Ibadan for PCR assay and systematically screened for the presence of HIV var genes.

G.Cytokine level assessments

Plasma samples were analyzed to assess cytokine levels. Levels of TNF-α, IL-2, IFN- γ, and IL-10 were determined using commercially available, ELISA kits (MabTECH, Sweden). Samples were titrated in duplicate, and the optical density at 405nm was read using an automatic microplate reader (Biotrack II). The concentration of cytokine in each sample was determined using a standard curve generated with each assay according to the manufacturer's instruction.

The questionnaire used was checked for content and face validities by experts in the Faculty of Education at the University of Ibadan. A pilot test was carried out on fifty pregnant volunteers after necessary corrections had been effected, in order to establish the reliability of the questionnaire. Structured questionnaires were use to collect demographic data (e.g. Age, Sex, Family size etc), Clinical data/History, Last menstrual date, estimated date of delivery etc.

H.Ethics of the Study

The study was approved by the Joint Ethical Committee UI/UCH and the concerned hospitals. Other ethical considerations were obeyed as contained in the National code of Health research ethics [11] . Each respondent was counselled before and after informed consent was given.

III. RESULTS

The HIV status of the respondents is shown in Table 1. A positive sero-prevalence for mothers 43/147(35.6%) and infants 19/30 (10.7%) was observed. Malaria prevalence rates at recruitment were higher (Table 2), at 57.0% for mothers and 63.3% for infants. 44.4% of mothers were co-infected in the study (Table 3). There was no association between HIV status and cytokine levels, (Table 4), however there was a significant relationship between HIV and the quantity of TNF-α, IL-2 and IFN-γ produced. There was no significant relationship between the concentration of cytokine and malaria infection (Table 5).

TABLE I
INCIDENCE OF HIV AMONG THE RESPONDENTS

HIV STATUS	Mother	Infants	Total
Positive	63 (35.6)	19 (10.7)	82 (46 .3)
Negative	83 (47.5)	11 (6.2)	95 (53 .7)
Total	147 (100.0)	30 (100.0)	177 (100.0)

TABLE II
MALARIA PARASITEMIA AMONG THE RESPONDENTS

Malaria	Mother	Infants	Total
Positive	85 (57.0)	19 (63.3)	104(58.1)
Negative	64 (43.0)	11 (36.7)	75(41.8)
Total	149 (100.0)	30 (100.0)	179 (100.0)

TABLE III
CO-INFECTION STATUS OF THE RESPONDENTS

	Malaria Status		
HIV Status	Negative	Positive	Total
Positive	39 (48.8)	43 (44.3)	82 (46.3)
Negative	41 (51.2)	54 (55.7)	95 (33.7)
Total	80 (100.0)	97 (100.0)	177 (100.0)

TABLE IV
ASSOCIATION BETWEEN HIV STATUS AND CYTOKINE LEVELS

Cytokine profiles (pg/µL)	Positive	Negative	X±S.D (Std. Error)	P-value
TNF-α	81	94	25.1± 16.2(1.8)	
			16.4± 11.2(1.2)	0.0000*
IL-2	75	90	51.9± 26.9(26.3.1)	
			79.6 + 24.5 (2.6)	0.0000*
IL-10	82	95	32.1± 5.06(0.6)	
			31.4± 4.3(0.4)	0.314+
IFN-γ	77	82	25.7± 25.5(2.9)	
			42.5±32.1(3.6)	0.0000*

* Significant, SD = Standard deviation, +Non significant

TABLE V
ASSOCIATION BETWEEN MALARIA STATUS AND CYTOKINE LEVELS

Cytokine Profiles (pg/µL)	Malaria Status			
	Positive	Negative	X±S.D (Std. Error)	P-value
TNF-α	96	79	19.2±13.8(1.4)	
			21.9±15.1(1.7)	0.210 +
IL-2	90	75	69.4±29.4(3.1)	
			64.2+28.5(3.3)	0.256+
IL-10	97	80	32.0±4.1(0.4)	
			31.6±5.2(0.6)	
0.345+				
IFN-γ	89	70	35.1±29.8(3.2)	
			33.2±30.1(3.7)	0.695+

* Significant, SD = Standard deviation, +Non significant

IV. DISCUSSION

Cytokines have been used as biomarkers to select pathophysiological processes [30] and may also serve as biochemical second messenger [14]. In this study, the concentration of the four cytokines, TNF-α, IL-2, IFN-γ and IL-10, investigated was used both as pathological and protective indicators. Among these, only IL-10 was of Th2 origin (anti- inflammatory) while TNF-α, IL-2 and IFN-γ were pro-inflammatory. There has been a strong association between levels of pro-inflammatory cytokines and spontaneous abortion [20, 9]. Several authors have attributed successful pregnancy to the intricate balance between pro-inflammatory and anti-inflammatory cytokines in both human [21, 27, 4, 5] and animal (murine) models. In the present study, we observed a predominantly reduced production of anti-inflammatory cytokine (IL-10), which corroborates earlier studies that suggested successful pregnancy is as a result of a shift of cellular immune responses (Th1) to antibody mediated immune response (Th2). This study reported high parasitaemia in the study population.

The prevalence of malaria parasites in HIV negative mothers 61.9% (52/34) corroborates several studies that have documented malaria as a major public health problem affecting between 300-500 million people annually[22, 32] and a major cause of maternal and infant morbidity and mortality in Sub-Saharan Africa [12, 29, 8,18]. This study is in consonance with the work of [22], reported malaria as a major threat to successful pregnancy due to prevailing Th 2 cytokine. Although they obtained blood from three measures, this included peripheral, placental smear and placental histology.

TNF-α, IFN-γ and IL-2 are soluble mediators of the cellular response; this study investigated peripheral concentration of three pro-inflammatory cytokines in consonance with the objective of this study and then compared its concentration with anti-inflammatory cytokine (IL-10) well known for its uses as a marker for inflammatory placental malaria [13, 14, 23].

The role of TNF-α in the pathogenesis of HIV in leading to increase in viral load in pregnant mothers in Cameroon had earlier been documented by [15]. This study observed nosignificant difference in TNF-α level between malarious mothers and their neonates thereby corroborating the work of [28]. However, this observation is worrisome because of its attending implication on foetal survival [27].

Among the malarious group (mother and infant), a high concentration of TNF-α was observed. This is in consonance with the work of [17], and could be indicative of viral replication. Interestingly, the concentrations of the other two pro-inflammatory cytokines (IL-2 and IFN-γ) in malarious neonates in this study could depict serious humoral perturbation as a result of mother-to-child transmission of malaria parasite [3,13] also reported undetectable levels of cytokine in samples from exposed infected infants. The study observed a discordant result between mothers that were HIV positive and malaria negative and their neonate counterparts. Among the mothers high levels of IL-2 and IFN-γ were observed, whereas high levels of all the three pro-inflammatory cytokines (IL-2, IFN-γ and TNF-α) were seen in neonates. However, no detectable changes in the plasma concentration of IL-10 were observed throughout the study in neonates.

The implication of this was that the immune system in neonates shifted to favour predominantly antibody response and reduction in the plasma concentration of IL-10. In HIV positive mothers we observed a predominant increase of pro-inflammatory response with no delectable change in the concentration of IL-10. This observation contradicts [23] who documented elevated plasma level of IL-10 and TNF-α but a reduced plasma concentration of TNF-γ and IL-2. Also several other groups [7, 19] variously demonstrated that HIV infection is associated with a switch from a predominantly T-helper type 1 (Th1) to T-helper type 2 (Th2) response: that is, a decreased production of IL-2 and IFN-γ, and increased level of IL-10 and TNF-α. Elevated plasma levels of IL-2 and IFN-γ in HIV- positive mothers selected from Saki differs from previous results [7]. In the malaria and HIV co-infection group, it was observed that while the plasma levels of IL-10 remained low throughout the course of the study, all the three pro-inflammatory cytokines (TNF-α, IL-2 and IFN-γ) was

elevated, thus making it Th1 dominant response.

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