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Upregulation of Peripheral Blood Levels of Immune-Regulatory Interleukin-10 Cytokine in Human Immunodeficiency Virus-infected Compared to Non-infected Pregnant Women

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ABSTRACT

Alterations in the cytokine status have been suggested to be at least partly responsible for the immune tolerance during pregnancy. Indeed, interleukin-10 (IL-10) cytokine is known to control inflammation-induced pathologies of pregnancy. The current study was set to investigate if HIV infection influences the production of IL-10 cytokine during pregnancy in adult asymptomatic HIV-infected pregnant women (n=44) and antiretroviral-naïve at enrolment as compared to controls (HIV non-infected pregnant women, n=44). Quantitative analysis of the IL-10 cytokines was done using Cytometry Bead Array Technology. Data analysis was performed using STATA version 13. P-value ≤ 0.05 was considered statistically significant. Results revealed differences in the mean of IL-10 cytokine levels between the HIV-positive and the HIV-negative participants over time across trimesters; trimester one 1.5 [(95% CI: 0.4, 2.5), P=0.008], second trimester 1.0 [(95% CI: 0.2, 1.9), P=0.019], and third trimester 1.2 [(95% CI: 0.3, 2.1), P=0.009]. These results demonstrates that, HIV infection favor the up regulation of IL-10 cytokine production in pregnancy. This upregulation of IL-10 may control inflammation-induced pathologies due to HIV during pregnancy. Further insight into the complexity of IL-10 cytokine in both HIV infections and pregnancy is greatly recommended to enable novel development of therapeutic strategies using this cytokine and targeting HIV-infected pregnant women.

Key Words: HIV infection, Pregnancy, Interleukin-10, Trimesters

INTRODUCTION

During human pregnancy, immune tolerance mechanisms are triggered to allow the i-allograft to develop and survive within the woman uterus in harmony with the immune system. Among other mechanisms, alterations in the cytokine status have been suggested to be at least partly responsible for the immune tolerance during pregnancy. Indeed, interleukin-10 (IL-10) is known in controlling inflammation-induced pathologies of pregnancy⁽¹⁾. IL-10 is a unique cytokine because it is an anti-inflammatory and immunosuppressive^(2,3). IL-10 plays an important role in pregnancy in immunomodulating the balance of anti-inflammatory and pro-inflammatory cytokines at the maternal-fetal interface⁽⁴⁾. The success of pregnancy is therefore associated with the production of IL-10 which inhibit the production and activity of Th1 cells, thus preventing loss of pregnancy⁽⁵⁾.

In respect to pregnancy, IL-10 levels upregulate markedly in the early months of pregnancy and remain high through to the last trimester just before start of labor⁽⁶⁾. Contrary to that, in HIV infection, there is a down-regulation of IL-10 activity^(7,8). The current study was set to understand if HIV infection influences the production of IL-10 cytokine during pregnancy in western Kenya. Findings from this study will help in the design of new strategies of immune intervention using this cytokine for HIV-infected pregnant women in this vulnerable group.

MATERIALS AND METHODS

Study population. The study evaluated 44 adult asymptomatic HIV-infected and antiretroviral-naïve pregnant women attending Academic Model for Providing Access to Healthcare (AMPATH) center and Moi Teaching and Referral Hospital (MTRH) - Mother Child Health Care (MCH) Clinics situated in western part of Kenya between June 2013 and June 2014. As controls, the study also included 44 HIV non-infected pregnant women. The history and complete physical and symptoms examinations were performed and

recorded at recruitment. Demographic and clinical data, including age, gestational age, last menstrual period, history of drug use, and number of births, was obtained from the medical record. The study participants recruited had CD4 T cell count equal or greater than 500 cells/ μ l. Women with any other infection besides HIV, who smoked or used illegal substances and with any complications in pregnancy (previous or present) were not included in the study.

Pregnant women who met the eligibility criteria in their first trimester and provided a written informed consent were enrolled. Subsequent study visits were scheduled in the second and third trimester. At each study visit, the study participants had blood (~5 ml) drawn for measurement of IL-10 cytokine levels. Quantitative analysis of the IL-10 cytokines was done using BD™ Cytometric Bead Array (CBA) Technology (BD Biosciences, San Jose, CA, USA). The lowest detection level of IL-10 cytokine on the CBA was 2.8 pg/ml.

Statistical analysis. Statistical analysis was performed using STATA version 13 (Statacorp, Timberlake, UK). Categorical variables of the study were reported as frequencies and percentages. Continuous variables of the study were reported as median and interquartile range (IQR) if they failed to satisfy the Gaussian assumption; otherwise mean and Standard Error of Mean (SEM). The assumption of normality was performed using Shapiro Wilks test. The association between categorical variables was done using Pearson's Chi Square test. The differences in the expression levels between continuous study variables was analyzed by Wilcoxon two sample test, if the variables did not satisfy the Gaussian assumptions. The change in the IL-10 cytokine levels in the first, second and third trimesters of advancing pregnancy was explored using repeated measures regression model. The changes were summarized with the corresponding 95% confidence intervals (95% CI). P -value ≤ 0.05 was considered statistically significant.

Ethical Consideration. This study obtained Institutional Review Board approval (#000915) from Moi Teaching and Referral Hospital Ethical Review Committee (ERC). The study participants also gave a voluntary written informed consent before enrollment and confidentiality of the study participants was maintained throughout the research period.

RESULTS

Demographic and baseline characteristics of the study participants. The demographic and baseline characteristics of the study participants were collected and summarized (Table 1). Forty four HIV-positive and equal HIV-negative participants were used as the study participants. The median age of the study participants was 28(IQR: 25-31) years. Thirty-six participants (41%) were at parity one, 37(42%) were at parity two, 12(14%) were at parity three and 3(3%) were at parity four. The median gestation age of the study participants was 8(7-9) weeks. The median age for the HIV-positive [29(IQR: 26-31)] participants was comparable to the HIV-negative [27(IQR: 24-30)] participants ($P=0.053$). Similarly, the differences in parity and gestational age between the HIV-negative and the HIV-positive participants were comparable ($P=0.058$ and $P=0.147$, respectively). At baseline, the mean IL-10 cytokine levels among the HIV-negative participants was significantly lower ($P=0.023$) than that of the HIV-positive participants, 1.6(IQR: 0-2.3) vs. 3.0(IQR: 0-4.3), pg/ml, respectively.

These findings demonstrate that despite similarities in age, parity and gestational age of the study groups, the baseline IL-10 cytokine levels was significantly lower in HIV-negative compared to the HIV-negative pregnant women.

Changes in IL-10 cytokine levels across the trimesters of HIV-positive and -negative pregnant women. In order to determine the mean changes of IL-10 cytokine levels across the trimesters of both HIV-positive and -negative pregnant women, the mean IL-10 cytokine levels

were measured at the first, second and third trimesters of advancing pregnancy (Table 2).

The mean changes in IL-10 cytokine levels across trimesters were assessed using repeated measures regression models. Among the HIV-positive and HIV negative participants, the mean IL-10 levels for the first, second and third trimesters were 2.9, 3.1, and 3.4 pg/ml, and 1.4, 2.1, and 2.2 pg/ml respectively. Among the HIV-positive, the mean change in IL-10 cytokine levels in the second trimesters was comparable to that of the first trimester, [0.2(95% CI: -0.4, 0.7), $P=0.554$] pg/ml. The mean change in IL-10 cytokine levels for the third trimester was comparable to that of the first trimester, [0.5(95% CI: -0.1, 1.0), $P=0.082$] and that of the second trimester, [0.3(95% CI: -0.2, 0.9), $P=0.258$]. Among the HIV-negative, the mean change in IL-10 cytokine levels during the second trimester was significantly higher than that of the first trimester, [0.7 (95% CI: 0.1, 1.2), $P=0.019$] pg/ml. The mean change in IL-10 cytokine levels for the third trimester, was higher than that of first trimester [0.8(95% CI: 0.2, 1.3) $P=0.008$] pg/ml but comparable to that of the second trimester, [0.1(95% CI: -0.5, 0.6) $P=0.745$] pg/ml.

The mean differences in IL-10 cytokine levels between HIV-positive and HIV-negative participants across the trimesters of pregnancy was also measured at the first, second and third trimesters of advancing pregnancy (Table 3). The differences in mean IL-10 cytokine levels between the HIV-positive and the HIV-negative participants over time was statistically significant in all trimesters, trimester one 1.5 [(95% CI: 0.4, 2.5), $P=0.008$], second trimester 1.0 [(95% CI: 0.2, 1.9), $P=0.019$], and third trimester 1.2 [(95% CI: 0.3, 2.1), $P=0.009$].

Collectively these results indicate that mean change in IL-10 cytokine levels among the HIV-negative increased significantly from the first to the second trimester. However, the HIV-positive showed comparable mean changes in IL-10 cytokine levels across the three trimesters. The present results also indicate that HIV-positive

pregnant group had significantly higher IL-10 cytokine levels in all trimesters compared to the HIV-negative pregnant group.

Table 1: Demographic and characteristics of the study participants at baseline

Variable	Levels	HIV-Positive (n=44)	HIV-Negative (n=44)	Total (n=88)	P
		N (%) or median (IQR)	N (%) or median (IQR)	n(%) or median (IQR)	
Age		29(26-31)	27(24-30)	28(25-31)	0.053
Births	One	12(27%)	24(55%)	36(41%)	0.058
	Two	23(52%)	14(32%)	37(42%)	
	Three	7(16%)	5(11%)	12(14%)	
	Four	2(5%)	1(2%)	3(3%)	
Gestation Age		8(7-8)	8(7-10)	8(7-9)	0.147
IL-10		3.0 (0-4.3)	1.6 (0-2.3)	1.6 (0-3.1)	0.023

Table Legend: The median age of the study participants was 28(IQR: 25-31) years. Thirty-six participants (41%) were at parity one, 37(42%) were at parity two, 12(14%) were at parity three and 3(3%) were at parity four. The median gestation age of the study participants was 8(7-9) weeks. The median age for the HIV-positive [29(IQR: 26-31)] participants was comparable to the HIV-negative [27(IQR: 24-30)] participants

($P=0.053$). Similarly, the differences in parity and gestational age between the HIV-negative and the HIV-positive participants were comparable ($P=0.058$ and $P=0.147$, respectively). At baseline, the IL-10 cytokine levels among the HIV-negative participants was significantly lower ($P=0.023$) than that of the HIV-positive participants, 1.6(IQR: 0-2.3) vs. 3.0(IQR: 0-4.3), pg/ml, respectively.

Table 2: Assessing change in IL-10 cytokine levels (pg/ml) across trimesters of HIV-positive and -negative pregnant women.

	HIV-Positive			HIV-Negative	
	Change (95% CI)			Change (95% CI)	
Trimester (Mean IL-10 pg/ml)	1 (2.9)	2 (3.1)	Trimester (Mean IL-10 pg/ml)	1 (1.4)	2 (2.1)
2 (3.1)	0.2(-0.4, 0.7) $P=0.554$	N/A	2 (2.1)	0.7(0.1, 1.2) $P=0.019$	N/A
3 (3.4)	0.5(-0.1, 1.0) $P=0.082$	0.3(-0.2, 0.9) $P=0.258$	3 (2.2)	0.8(0.2, 1.3) $P=0.008$	0.1(-0.5, 0.6) $P=0.745$

Table Legend: Among the HIV-positive and HIV negative participants, the mean IL-10 levels for the first, second and third trimesters were 2.9, 3.1, and 3.4 pg/ml, and 1.4, 2.1, and 2.2 pg/ml, respectively. Among the HIV-positive, the mean change in IL-10 cytokine levels in the second trimesters was comparable to that of the first trimester, [0.2(95% CI: -0.4, 0.7), $P=0.554$]

pg/ml. The mean change in IL-10 cytokine levels for the third trimester was comparable to that of the first trimester, [0.5(95% CI: -0.1, 1.0), $P=0.082$ and that of the second trimester, [0.3(95% CI: -0.2, 0.9), $P=0.258$]. Among the HIV-negative, the mean change in IL-10 cytokine levels during the second trimester was significantly higher than that of the first trimester,

[0.7 (95% CI: 0.1, 1.2), $P=0.019$] pg/ml. The mean change in IL-10 cytokine levels for the third trimester, was higher than that of first trimester

[0.8(95% CI: 0.2, 1.3) $P=0.008$] pg/ml but comparable to that of the second trimester, [0.1(95% CI: -0.5, 0.6) $P=0.745$] pg/ml.

Table 3: Test for differences in Interleukin-10 cytokine levels (pg/ml) between HIV-positive and HIV-negative participants across the trimesters of pregnancy.

Variable		HIV-positive	HIV-negative	Difference (95% CL)	
	Trimester	mean(SEM)	mean(SEM)	(Mean HIV+) - (Mean HIV-)	<i>P</i>
IL-10	1	2.9(3.3)	1.4(1.2)	1.5(0.4, 2.5)	0.008
	2	3.1(2.3)	2.1(1.5)	1.0(0.2, 1.9)	0.019
	3	3.4(2.5)	2.2(1.6)	1.2(0.3, 2.1)	0.009

Table 3 legend: The differences in mean IL-10 cytokine levels between the HIV-positive and the HIV-negative participants over time was statistically significant in all trimesters, trimester one 1.5 [(95% CI: 0.4, 2.5), $P=0.008$], second trimester 1.0 [(95% CI: 0.2, 1.9), $P=0.019$], and third trimester 1.2 [(95% CI: 0.3, 2.1), $P=0.009$].

DISCUSSION

The present study was designed to investigate the effect of HIV infection on peripheral blood levels of immune-regulatory IL-10 cytokine in advancing pregnancy. Comparing the mean changes and differences of IL-10 cytokine levels between the HIV-infected and non-infected pregnant women, it was observed IL-10 cytokine levels to be higher in the HIV-infected group than the non-infected. The current study therefore demonstrates an increasing trend of IL-10, a key type 2 cytokine, in advancing pregnancies with or without HIV infection. These results support the notion that IL-10 cytokine is up-regulated and is critical in pregnancy. Previous studies have observed that IL-10 cytokine can inhibit pro-inflammatory activities and down-regulate tissue disruptions as a result of inflammatory activities⁽⁹⁾ due to HIV infection and pregnancy in all trimesters⁽¹⁰⁾. These observations explain the continuous increase of IL-10 cytokine overtime in an advancing pregnancy of both HIV-infected and uninfected pregnant women according to the present study results.

In other related studies in support of the present findings, it was observed that there was a significantly higher *in vitro* production of IL-10 cytokine by PBMCs during pregnancy than 2 years after⁽¹¹⁾ and higher levels in early pregnancy⁽¹²⁾ and last trimester of pregnancy⁽¹³⁾ and at delivery⁽¹⁴⁾. In support of the present findings and in the context of conjoint cases of HIV and pregnancy, higher levels of IL-10 cytokines were produced by activated T-cell cultures from HIV-infected pregnant women compared to their controls⁽¹⁵⁾. Similarly in another study, higher production of IL-10 cytokine levels were obtained in T-cell cultures from pregnant women following addition of HIV antigens⁽¹⁶⁾. These observations could be attributed to the fact that pregnant women produce IL-10 cytokine in high levels to assist them control the HIV replication, and reduce the risk of vertical HIV transmission⁽¹⁵⁾. The secretion of high levels of IL-10 therefore seems to be vital in enhancing the success of pregnancy in both cases of HIV-infected and non-infected women. The higher IL-10 cytokines levels among the HIV-infected pregnant women seem to be as result of the synergic effect of both pregnancy and HIV infection.

CONCLUSION AND RECOMMENDATION

These results demonstrates that HIV infection favors the up-regulation of IL-10 production in pregnancy. Due to the perturbations to cytokines

activity, further insight into the complexity of IL-10 cytokine in both HIV infections and pregnancy is greatly recommended to enable novel development of therapeutic strategies using this cytokine and targeting HIV-infected pregnant women.

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