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Full Length Research Article

Midgestational Characterization of Cytokine Profiles in HIV Infected and Uninfected Black South African Pregnant Women

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Summary: Pregnancy is characterized by an anti-inflammatory milieu in the second trimester despite a pro-inflammatory response in the first and third trimesters. Nonetheless a disproportionate inflammatory response is risky in pregnancy. This retrospective study evaluated the mid-gestational expression of inflammatory and anti-inflammatory cytokines in HIV infected pregnant women at their first antenatal visit. Archived serum samples were collected from seventy (n=70) black pregnant women, attending a primary health care centre in KwaZulu-Natal, South Africa. The demographic and clinical profiles were procured from patient medical records and cytokine levels were measured in all samples. A statistically significant difference was noted for IP-10 ($p<0.05$) between the HIV positive and HIV negative groups. Likewise, a statistically significant difference was observed for IL-7 in the HIV population, when further stratified based on ART usuage. Significant correlations were noted between IL-7 and birthweight ($r=0.35$, $p<0.05$); IFN-δ and maternal age ($r=-0.27$, $p<0.05$); TNF-α and gestational age ($r=0.26$, $p<0.05$); VEGF and systolic blood pressure ($r=0.40$, $p<0.05$); IL-4 and gestational age ($r=-0.30$, $p<0.05$). A positive correlation was noted for inflammatory IL-1b with anti-inflammatory IL-5; IL-5 and FGF basic; inflammatory IL-2 with anti-inflammatory IL-5, IL-10 and FGF basic. A negative correlation was noted between the inflammatory IL-12 with anti-inflammatory IL-1ra and Il-4; and between IL-17A with IL-10. This study reveals midgestational variation in serum inflammatory and anti-inflammatory immunologic profile of pregnant women, irrespective of the use of antiretroviral therapy. This disparity in the susceptible HIV infected women will affect progression of pregnancy and encourage fetal morbidity and mortality.

Keywords*: inflammatory, anti-inflammatory, cytokines, interleukins, pregnancy*

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INTRODUCTION

During the pathogenic process of HIV replication, cytokines regulate the innate and adaptive immune response (Clerici, 2010). Moreover, they also influence reproductive function such as follicle growth, blastocyst implantation, trophoblast growth and parturition (Bowen *et al*., 2002). The physiologic control of innate immunity during pregnancy is required to support the acceptance of the fetal allograft. The switch from the T helper (Th)1 (cell-mediated response) to Th2 (humoral response) facilitate pregnancy success (Challis *et al*., 2009; Mosmann & Sad, 1996; Raphael *et al*., 2015). This feto-maternal rapport is safeguarded by the balance created by the Th1/Th2 activity in response to the placental production of cytokines (Challis *et al*., 2009; Robertson *et al*., 1994). More specifically, placental trophoblasts secrete pro-inflammatory cytokines (Szekeres-Bartho & Wegmann, 1996), whilst the anti-inflammatory cytokines are secreted by tissues (Joachim *et al*., 2003), both of which are central to trophoblast/angiogenic function (Omar *et al*., 2005). Pregnancy is thus characterized by an anti-inflammatory milieu in the second trimester despite a pro-inflammatory response in the first and third trimesters (Marie-Pierre Piccinni *et al*., 2015). Nonetheless a disproportionate inflammatory response is risky in pregnancy (Orsi & Tribe, 2008).

The Th1-response stimulates macrophages and cell mediated immunity through secretion of interleukins (IL-1, IL-2, IL-6, IL-12, IL-15, IL-18), interferons (IFN-gamma) and tumor necrosis factor alpha (TNF-α) (Challis *et al*., 2009; Marie-Pierre Piccinni *et al*., 2015). The Th2-response in contrast accelerates humoral immunity in response to secretion of IL-4, IL-5, IL-10, IL-13, and granulocyte macrophage colony stimulating factor (GM-CSF) (Challis *et al*., 2009; Marie-Pierre Piccinni *et al*., 2015). Infections or inflammatory events are known to disrupt this balance causing a dominant Th1 control, which is believed to increase the inflammatory cytokine production (Orsi & Tribe, 2008). Increased levels of serum pro-inflammatory cytokines are known to be a risk factor for preterm delivery by causing premature uterine contraction and membrane rupture (Murtha *et al*., 2007). This maternal immunomodulation is also transmuted in pregnancy pathologies such as preeclampsia and intrauterine growth restriction resulting in a higher Th1 response (Hu *et al*., 2007; Orsi & Tribe, 2008).

HIV infection prevalence rates in South African females of childbearing age, is approximately 22.8% (Statistics South Africa, 2015), with this prevalence being greater amid pregnant females (National Department of Health, 2013). With the recent rollout and increase in ART access, almost 95% of HIV-infected pregnant women receive triple drug antiretroviral therapy (ART) during pregnancy and breast feeding (United Nations Programme on HIV/AIDS, 2017). Whilst ART decreases HIV related maternal morbidity and mortality, it also demonstrates a characteristic shift from a Th1 to Th2 immune response in HIV treated pregnancies (Osakwe *et al*., 2010; Marie-Pierre Piccinni *et al*., 2015). This immune restorative effect of antiretroviral therapy normalizes the shift in normal pregnancy (Fiore *et al*., 2006). Based on the reduced morbidity and mortality observed in pregnant women on ART for prevention of mother to child transmission (PMTCT), a dual antiretroviral program was endorsed by the South African National Department of Health, as the standard of care for PMTCT regardless of the infection stage or immunological status of the pregnant women (Moodley *et al*., 2016; National Department of Health, 2008). Dual ART exposure is also linked with reduced odds for adverse birth outcomes (Moodley *et al*., 2016).

HAART-mediated endothelial dysfunction has been correlated with elevated blood pressure in HIV-positive patients (Seaberg *et al*., 2005; Stein, 2003). A recent meta-analysis revealed that systolic and diastolic blood pressure levels and hypertension risk are significantly increased in response to HAART treatment (Nduka *et al*., 2016). This corroborates the study done by Bergersen and co-workers in a Norwegian cohort of HIV-positive patients receiving HAART (Bergersen *et al*., 2003). Their study indicated that the incidence of hypertension was increased amongst those on HAART in comparison to those not on HAART (Bergersen *et al*., 2003).

At present there is conflicting data concerning BMI and weight gain amongst HIV patients on HAART (Amorosa *et al*., 2005; Crum-Cianflone *et al*., 2008; Gallant *et al*., 2004; Mallon *et al*., 2003; Sharma *et al*., 2014; Shikuma *et al*., 2004). Despite a high prevalence of overweight and obese women in their cohort of HIV infected pregnancies, Sharma *et al*., (2014) demonstrated only a small increase in BMI over the course of pregnancy (Sharma *et al*., 2014). Pregnancy is identified as an immune process and may be especially disturbed by HAART use (Sebitloane & Moodley, 2017). Limited South African studies are available that characterize the cytokine profiles during pregnancy and its link with HAART and adverse birth outcomes. This study therefore evaluated the mid-gestational serum levels of both inflammatory and anti-inflammatory cytokines in HIV+ve and HIV-ve Black South African pregnant women. We also provide an epidemiological correlation between the cytokines and BMI, BP, hemoglobin and birth weight.

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MATERIALS AND METHODS

Study population and sample collection: This was a retrospective study, which used archived serum samples collected from seventy (n=70) Black pregnant women attending a primary health care centre in KwaZulu-Natal, South Africa. The demographic and clinical profiles of the study population were obtained from patient medical records. Anemia in pregnancy, was defined as a hemoglobin level less than 11.0 g/dl (World Health Organization, 2011). Serum samples were collected at their first antenatal booking, between 10-20 weeks' gestation. Circulating cytokine levels were measured in all serum samples collected. All samples were stratified by HIV status into HIV-ve and HIV+ve normotensive pregnant groups.

Pregnant women with underlying medical conditions and those unable to provide informed consent were excluded from the study.

Ethical consideration: Ethical approval was obtained from the Durban University of Technology Institutional Research Ethics Committee (IREC 010/17) and the KZN Department of Health.

Quantification of analytes: The cytokine expression was determined using the Bio-Plex Pro Human Cytokine Group I Assays panel kit (cat no: M500KCAF0Y, Bio-Rad, USA), in accordance to the manufacturer's protocol (www.bioplex.com/bioplex). All reagents and samples were brought to room temperature before use. Coupled magnetic beads (Bio-Rad) were dispensed into each well of the assay plate, followed by washing. Standards and samples (1:4) were diluted, vortexed and incubated for 1 hour at room temperature with vigorous shaking at 850±50 rpm. Post incubation, plates were washed and detection antibodies were incubated for 30 minutes at room temperature. Streptavidin-PE (1x) was added to each well and incubated in the dark at room temperature for 10 min at 850±50rpm. Plates were washed and re-suspended in assay buffer at 850±50 rpm for 30 seconds and then read on a Bio-Plex Pro MAGPIX system. The serum values for each cytokine was generated using the Bio-Plex Manager software (version 6.1, Bio-Rad).

Data analysis: STATA (version 12, STATACORP) was used for data analysis. Data is presented as mean and standard deviation for continuous data and frequency distributions for categorical variables. The Pearson's chi-squared test was used to evaluate bivariate associations between demographic and clinical variables stratified by HIV status. HIV status was evaluated as a binary variable (HIV Positive (+) vs HIV Negative (-). Similar bivariate analysis was used to compare clinical characteristics stratified by the use of antiretroviral therapy (HAART). As the distributions of measured cytokines were not normally distributed, we performed a log-transformation to normalize data and geometric means were compared by HIV status and use of HAART. Pearson's correlation was used to assess the relationship between inflammatory and anti-inflammatory cytokines. Correlation was also used to estimate whether levels of cytokines depended on maternal epidemiological and clinical characteristics. A p-value ≤ 0.05 was considered statistically significant at a 95% confidence level.

RESULTS

Participant demographics: The demographic profile of the study population, stratified by HIV status is shown in Table 1. The mean age of the HIV+ve and HIV-ve study groups

was 26.89±5.07 and 24.54±5.02 yrs respectively. Of the total participants enrolled, 41 (59%) were HIV+ve, of which 23 (56%) received antiretroviral treatment (Table 1). A significant difference was noted for gravida between the HIV+ve and HIV-ve study groups.

Table 1:
Demographic and clinical profile of the study population (N=70)[#]

		HIV+ve (n=41)	HIV-ve (n=29)	P value
Age (years) (mean, SD)		26.89 (5.07)	24.54 (5.02)	
PMTC (n, %)		23 (56%)	n/a	
Parity (n, %)	*Nulliparous*	8 (21.05)	12 (50.00)	0.06
	Primiparous	15 (39.47)	9 (37.50)	
	Multiparous	15 (39.47)	3 (12.50)	
***Gravida** (n, %)	*primigravida*	7 (18.42)	11 (45.83)	0.05
	multigravidaє	31 (81.57)	13 (54.17)	
***Gestational age at booking** (wks, mean±SD)		15.44 (5.31)	12.22 (5.27)	0.03
BMI (kg/m^2, mean±SD)		29.58 (10.9)	26.63 (3.94)	0.34
Blood pressure (mmHg, mean±SD)	*Systolic*	108.38 (13.73)	112.13 (14.69)	0.49
	Diastolic	62.85 (9.34)	66.4 (6.31)	0.24
Hemoglobin (g/dl, mean±SD)		10.63 (3.41)	11.39 (2.10)	0.48
Birthweight (kg, mean±SD)		3.10 (0.44)	3.18 (0.65)	0.65

**p<0.05 was considered statistically significant*
[#] data was missing in certain categories

Table 2:
Clinical characteristics based on antiretroviral therapy status

HIV Treatment Regimen (n=41)			
	HAART use (n=23)	**No HAART (n=18)**	***p*-value**
Body Mass Index (kg^2, mean±SD)	29.45 (8.25)	29.67 (13.13)	0.97
Blood pressure (mmHg, mean±SD)			
Systolic	114.83 (17.66)	102.86 (6.31)	0.12
Diastolic	67.83 (8.61)	58.57 (8.16)	0.07
Haemoglobin (g/dl)	12.35 (2.15)	9.16 (3.73)	0.09
Birthweight (kg)	3.09 (0.44)	3.13 (0.48)	0.89
***p<0.05 was considered statistically significant**			

Values are expressed as mean±SD

Gestational age was significantly different (p=0.03) between the HIV+ve and -ve groups. The BMI was greater within the HIV+ve group compared to the HIV-ve group (Table 1). In contrast, both the mean systolic and diastolic blood pressure was lower in the HIV+ve group compared to the HIV-ve group. The hemoglobin levels were also higher in the HIV negative group compared with the HIV positive group. None of the participants smoked and only 1 consumed alcohol. This data was therefore excluded from further analyses.

Bivariate analyses between selected clinical factors and the use of antiretroviral therapy (ART) is shown in Table 2. No statistically significant difference was observed for any of the factors. However, of note, hemoglobin was elevated amongst those receiving dual ART compared to ARV naïve patients. Additionally, both systolic and diastolic mean blood pressures were higher amongst those on dual ART exposure in contrast to those not.

Cytokine expression: The mean± standard deviation for the measured cytokines are presented in Table 3. There were no statistically significant differences noted between the HIV-positive and HIV-negative groups for both the inflammatory and anti-inflammatory cytokines as well as chemokines when stratified by HIV status, except for inflammatory IP-10 (p<0.05). Similarly, when the HIV population was stratified based on ART usage, only the inflammatory IL-7 was statistically different (p<0.05).

Correlations between inflammatory/anti-inflammatory cytokine expressions and epidemiological factors: The Pearson correlation was used to compare associations between both the inflammatory/ anti-inflammatory cytokines with selected epidemiological factors (Table 4). Our data demonstrates significant correlations between IL-7 and birthweight (r=0.35, p<0.05); IFN-δ and maternal age (r=-0.27, p<0.05); TNF-α and gestational age (r=0.26, p<0.05); VEGF and systolic blood pressure (r=0.40, p<0.05); IL-4 and gestational age (r=-0.30, p<0.05).

Correlations between inflammatory and anti-inflammatory cytokine expressions: The Pearson correlation revealed significant associations between pro- vs anti-inflammatory cytokines (Table 5). A positive correlation was noted for inflammatory IL-1b with anti-inflammatory IL-5, IL-5 and FGF basic; inflammatory IL-2 with anti-inflammatory IL-5, IL-10 as well as FGF basic (Table 5). A negative correlation between the inflammatory IL-12 with anti-inflammatory IL-1ra and Il-4 as well as that between IL-17A with IL-10 (Table 5) was noted.

DISCUSSION

Our study evaluated the mid-gestational expression of selected inflammatory and anti-inflammatory cytokines as well as chemokines in HIV infected pregnant women at their first antenatal visit. Pregnancy in HIV-infected women, poses an additional challenge to the already interrupted immune system. In light of the pervasive burden of HIV infection and the enormous ARV rollout in SA, the immune reconstitution effects of HAART during pregnancy may also impact the expression of both the inflammatory and anti-inflammatory cytokines (Maharaj *et al*., 2017).

Our data suggests that gestational age was significantly different between the HIV+ve and -ve groups. In South Africa, antenatal care is usually initiated before 20 weeks of gestation as prescribed by the National Department of Health (National Department of Health, 2013). It is possible that the HIV positive women sought antenatal care later than the HIV negative group, reflective of possible anxiety associated with mandatory HIV testing at the first antenatal visit.

Table 3:
Cytokine profiles of study population stratified by HIV status –log transformed #

Cytokines	Total sample n = 70	Total sample		HIV +ve (n=41)	
		HIV+ve (n=41)	HIV-ve (n=29)	HAART use (n=23)	No HAART (n=18)
Inflammatory					
IL-1b	7.20 (0.54)	7.18 (0.53)	7.18 (0.54)	7.22 (0.51)	7.20 (0.63)
IL-2	7.41 (0.08)	7.42 (0.00)	7.39 (0.14)	7.42 (0.00)	7.42 (0.00)
IL-6	6.16 (0.57)	6.13 (0.57)	6.16 (0.60)	6.11 (0.51)	6.15 (0.62)
IL-7	6.44 (1.36)	6.36 (1.28)	6.41 (1.47)	**5.95 (1.13)**	**6.89 (1.33)***
IL-8	5.47 (0.49)	5.46 (0.46)	5.51 (0.53)	5.52 (0.41)	5.34 (0.55)
IL-12(p70)	9.05 (0.67)	9.05 (0.74)	9.07 (0.56)	8.92 (0.97)	9.14 (0.30)
IL-15	5.59 (0.28)	5.56 (0.35)	5.63 (0.14)	5.53 (0.41)	5.60 (0.24)
IL-17A	7.63 (2.05)	7.36 (2.12)	8.17 (1.75)	7.71 (1.98)	6.78 (2.30)
Eotaxin	4.69 (1.55)	4.70 (1.60)	4.69 (1.63)	4.56 (1.31)	4.81 (1.81)
GM-CSF	8.91 (0.93)	8.90 (0.96)	8.98 (0.84)	8.99 (0.77)	8.69 (1.22)
IFN-g	3.63 (0.38)	3.62 (0.37)	3.62 (0.44)	3.61 (0.34)	3.65 (0.38)
IP-10	1.86 (1.10)	**1.68 (1.03)**	**2.28 (1.08)***	1.77 (1.16)	1.43 (0.92)
MCP-1(MCAF)	7.21 (2.10)	7.02 (2.06)	7.61(2.18)	6.91 (2.01)	6.91 (2.11)
MIP-1a	0.64 (1.39)	0.53 (1.37)	0.91 (1.48)	0.68 (1.33)	0.25 (1.36)
PDGF-bb	1.70 (1.49)	1.60 (1.62)	1.86 (1.04)	1.66 (1.57)	1.56 (1.91)
MIP-1b	5.41 (1.71)	5.44 (1.78)	5.61 (1.68)	5.53 (1.69)	5.09 (1.88)
RANTES	2.51 (0.86)	2.54 (0.88)	2.60 (0.51)	2.51 (1.06)	2.38 (0.98)
TNF-a	5.38 (1.46)	5.36 (1.56)	5.43 (1.38)	5.21 (1.48)	5.43 (1.61)
VEGF	7.97 (1.63)	7.82 (1.61)	8.12 (1.68)	7.89 (1.70)	7.77 (1.59)
Anti-inflam					
IL-1ra	4.49 (1.24)	4.54 (1.30)	4.43 (1.27)	4.82 (1.50)	4.19 (0.85)
IL-4	6.35 (0.31)	6.34 (0.34)	6.37 (0.27)	6.35 (0.31)	6.33 (0.37)
IL-5	7.23 (1.51)	7.20 (1.44)	7.18 (1.67)	7.11 (1.51)	7.33 (1.37)
IL-10	6.50 (1.78)	6.37 (1.67)	6.20 (2.04)	5.80 (2.07)	7.32 (0.92)
FGF basic	6.16 (1.03)	6.12 (0.98)	6.16 (1.16)	6.36 (0.87)	5.88 (1.04)
G-CSF	4.34 (0.66)	4.25 (0.61)	4.47 (0.80)	4.33 (0.73)	4.18 (0.38)
Chemokines					
IL-8	5.47 (0.49)	5.46 (0.46)	5.51 (0.53)	5.52 (0.41)	5.34 (0.55)
Eotaxin	4.69 (1.55)	4.70 (1.60)	4.69 (1.63)	4.56 (1.31)	4.81 (1.81)
IP-10	1.86 (1.10)	**1.68 (1.03)**	**2.28 (1.08) ***	1.77 (1.16)	1.43 (0.92)
MCP-1(MCAF)	7.21 (2.10)	7.02 (2.06)	7.61(2.18)	6.91 (2.01)	6.91 (2.11)
MIP-1a	0.64 (1.39)	0.53 (1.37)	0.91 (1.48)	0.68 (1.33)	0.25 (1.36)
MIP-1b	5.41 (1.71)	5.44 (1.78)	5.61 (1.68)	5.53 (1.69)	5.09 (1.88)
RANTES	2.51 (0.86)	2.54 (0.88)	2.60 (0.51)	2.51 (1.06)	2.38 (0.98)

** p < 0.05 was considered statistically significant*
 # Data for cytokine levels were log transformed

This is corroborated by a recent study which indicated that both knowledge and timing of a new diagnosis of HIV infection at the first antenatal visit places women at an increased risk for the development of depression during pregnancy (Nydoo *et al*., 2017). The burden of HIV/AIDS diagnosis, combined with an increased risk for the development of depression may adversely affect both mother and child health outcomes (Iyun *et al*., 2018; Moodley *et al*., 2016; Ramirez-Avila *et al*., 2012; Rochat *et al*., 2013).

Despite the implementation of the 2014 new ARV South African guidelines as a means to improve antiretroviral treatment coverage for all HIV positive pregnant women, there are still pregnant women who are unregistered for antenatal care, with unknown HIV status (Iyun *et al*., 2018; Moodley *et al*., 2016). Earlier studies have also suggested that being younger with minimal education, single with no partner support, and low socioeconomic status as potential reasons for being unregistered for antenatal care and thus being categorized as untreated HIV infected women (Fawcus & SR, 1992).

Despite the lack of statistical significance, our study demonstrates a trend in both the systolic and diastolic blood pressure between those receiving HAART compared to those not receiving HAART. Higher blood pressure is known to be elevated in patients receiving ART as it induces vascular endothelial alterations (Chow *et al*., 2003; Nduka *et al*., 2016). A recent systematic analysis confirmed that due to data deficiency, it is unclear if the distinctive effect of ART exposure on the risk of developing hypertension is due to the regimen of the ART or duration (Nduka *et al*., 2016). It is also possible that antiretroviral drugs compromise the production of vasodilatory molecules such as nitric oxide (Nduka *et al*., 2016). However, an earlier report also suggests that these elevations in blood pressure amongst HIV infected individuals, may arise in response to an exaggerated immune response subsequent to the use of antiretroviral drugs (Bosamiya, 2011). Nonetheless, the use of ART is linked to antiretroviral-associated hypertension, a phenomenon that is rapidly gaining momentum in becoming a major healthcare burden (Nduka *et al*., 2016).

Table 4:
Pearson's Correlation between cytokine profiles and epidemiological factors

Cytokines	Age	Gestational Age (wks)	BMI (kg/m^2)	Blood pressure (mmHg)		Hemoglobin (g/dl)	Birth weight (kg)
				Systolic	Diastolic		
Inflammatory							
IL-1b	-0.11	-0.08	0.08	0.23	0.15	-0.15	0.24
IL-2	0.01	-0.19	0.01	-0.11	0.03	0.16	0.23
IL-6	-0.04	-0.06	0.29	0.26	0.34	0.03	-0.05
IL-7	-0.04	-0.07	-0.10	-0.08	-0.01	0.06	**0.35***
IL-12(p70)	0.01	0.23	0.01	-0.11	0.03	0.16	0.10
IL-15	0.05	-0.07	-0.00	-0.03	0.01	0.24	0.09
IL-17A	-0.13	-0.02	-0.01	0.05	-0.15	-0.29	0.06
GM-CSF	-0.25	0.08	-0.06	0.05	-0.20	-0.35	-0.11
IFN-g	**-0.27***	-0.14	0.24	0.35	0.23	-0.16	0.08
PDGF-bb	-0.12	0.08	-0.03	0.08	0.14	0.03	0.08
TNF-a	-0.06	**0.26***	0.18	0.09	-0.16	-0.16	-0.02
VEGF	-0.24	0.08	0.35	**0.40***	0.32	-0.01	-0.02
Anti-inflam							
IL-1ra	-0.23	-0.01	0.14	0.11	0.16	-0.17	0.08
IL-4	-0.18	**-0.30***	0.21	0.24	0.28	-0.02	0.32
IL-5	-0.10	-0.18	0.07	0.10	0.20	0.22	0.20
IL-10	0.17	0.05	-0.07	0.08	0.15	0.17	-0.00
FGF basic	-0.19	-0.18	0.11	0.14	-0.10	-0.17	-0.11
G-CSF	-0.12	-0.04	-0.07	0.20	0.11	-0.09	0.08
Chemokines							
IL-8	-0.14	-0.05	0.09	0.274	0.15	-0.15	0.08
Eotaxin	-0.08	-0.09	-0.09	-0.002	-0.13	-0.18	-0.30
IP-10	-0.22	0.12	0.07	0.103	-0.02	-0.01	0.00
MCP-1(MCAF)	0.04	-0.06	-0.12	0.255	0.07	-0.23	0.12
MIP-1a	-0.01	0.20	-0.06	-0.007	0.03	-0.02	0.29
MIP-1b	-0.15	0.19	-0.21	-0.039	0.11	-0.01	0.09
RANTES	-0.09	0.05	-0.13	-0.156	-0.14	-0.25	0.16

**p<0.05 was considered statistically significant*

Table 5:
Pearson's correlation between Inflammatory and anti-inflammatory cytokines

		Anti-inflammatory cytokines					
		IL-1ra	**IL-4**	**IL-5**	**IL-10**	**FGF basic**	**GM-CSF**
Inflammatory	**IL-1b**	**0.69****	**0.72****	**0.33***	0.04	**0.29***	0.14
	IL-2	0.17	0.19	**0.81****	**0.53****	**0.34***	0.02
	IL-6	**0.84****	**0.67****	**0.50****	0.06	**0.43****	**0.41***
	IL-7	**0.25***	**0.31***	**0.71****	**0.52****	**0.30***	0.07
	IL-12(p70)	-0.01	-0.13	0.22	0.20	0.23	0.06
	IL-15	0.21	0.17	0.18	**0.25***	0.03	0.20
	IL-17A	**0.44****	**0.49****	0.15	-0.00	**0.41****	**0.43****
	GM-CSF	**0.53****	0.23	0.20	0.08	**0.58****	1.00
	IFN-g	**0.76****	**0.71****	**0.47****	0.07	**0.49****	**0.31***
	PDGF-bb	**0.39***	**0.50****	0.04	-0.09	0.13	**0.30***
	TNF-a	0.03	-0.11	0.05	0.04	**0.27***	0.16
	VEGF	**0.45****	**0.33***	**0.25***	0.04	**0.50****	**0.32***
Chemokines	**IL-8**	**0.55****	**0.50****	**0.24***	0.07	**0.40***	**0.25***
	Eotaxin	**0.39***	**0.31***	0.23	0.03	**0.34***	0.19
	IP-10	**0.38***	0.20	0.11	-0.03	**0.52****	**0.67****
	MCP-1(MCAF)	**0.25***	**0.25***	0.07	0.07	**0.53****	**0.41***
	MIP-1a	0.16	0.17	-0.08	-0.05	-0.04	0.02
	MIP-1b	**0.32***	**0.29***	0.07	-0.09	**0.27***	**0.40***
	RANTES	**0.28***	0.20	0.04	-0.01	**0.27***	**0.42****

**p<0.05 was considered statistically significant*
*** p<0.001 was considered statistically significant*

We also demonstrate a significant correlation between vascular endothelial growth factor (VEGF) and systolic blood pressure. Vascular Endothelial Growth Factor regulates angiogenesis, maintaining vascular homeostasis and permeability (Ghazizadeh *et al*., 2017). It is also responsible in maintaining endothelial function and blood pressure by stimulating NO synthase expression and the release of vasodilatory nitric oxide and prostacyclin (Ylä-Herttuala *et al*., 2007). However, elevated VEGF concentration is reported to physiologically predispose one to a greater risk of emergent cardiovascular anomalies (Ghazizadeh *et al*., 2017). This risk is amplified as a result of its release from foam cells and macrophages which stimulates the development of atherosclerosis (Ghazizadeh *et al*., 2017), combined with reports that the HIV accessory protein *tat* mimics VEGF increasing angiogenesis, thus supporting its powerful angiogenic effects (Barillari *et al*., 1999).

The anti-inflammatory IL-4 and IL-10 cytokines are instrumental in resolving inflammation during pregnancy (Chatterjee *et al*., 2014). Our data demonstrates statistically significant positive correlations between IL-4 and several of the inflammatory cytokines measured, including IL-1b, IL-6, IL-7, IL-8, IFN-G. In addition, we demonstrate an inverse association between IL-4 and IL-12, which corroborates Ouyang *et al*., (1998), who previously suggested that the Th2 immune response is strengthened by IL-4 due to suppression of the Th1 immune activity and IL-12 signaling (Ouyang *et al*., 1998). The anti-inflammatory effects of IL-10 is achieved due to the blockage of the inflammatory effects of IL-1, IL-6, IL-12, TNF and chemokines (Saraiva & O'Garra, 2010). Our data is similar in that it also demonstrates an inverse relation between IL-10 and the chemokines IP-10, MIP-1a, MIP-1b and RANTES, endorsing the anti-inflammatory effects required for pregnancy success. Thus, balance in IL-4 and IL-10 is essential in controlling the maternal inflammatory response, thereby endorsing the crucial crosstalk between the placenta and the fetus during pregnancy (Thaxton & Sharma, 2010). In the absence of this resolution by the anti-inflammatory cytokines, various pregnancy related anomalies may prevail which can jeopardize both maternal and neonatal health (Chatterjee *et al*., 2014). Ferguson *et al*., (2014) reiterates this finding, suggesting that maternal inflammatory cytokines such IL-6 and IL-10 may be related with a greater risk of spontaneous preterm birth, and placentally mediated preterm birth respectively (Ferguson *et al*., 2014). Likewise, cytokines IL-8, IFNγ, and TNFα are also reported to be associated with preeclampsia development and intrauterine growth restriction that is coupled with deficient placental function (Raghupathy *et al*., 2012).

The global increase in HIV-infected young women is associated with disruptions in the maternal immune status which subsequently disrupts the neonatal immune status (Kasahara *et al*., 2013). Despite the obvious anti-retroviral associated reduction in perinatal transmission, antiretroviral therapy during pregnancy is associated with a greater risk of premature births (Fiore *et al*., 2006). Our data demonstrates a reduction in IL-10 levels amongst the treated HIV+ve women in contrast to untreated women. Despite the lack of statistical significance, we also demonstrate and inverse relationship between IL-10 and birthweight. More recently, it was shown that low serum levels of IL-10 between 22 to 25 weeks' gestation, may be correlated with the risk of preterm birth in primigravidae (de Brito Pereira *et al*., 2016).

We also report a statistically significant difference in IL-7 levels between the HIV+ve cohort on ARV treatment compared to those not. Birth weight was not different between the latter 2 groups, however, a statistically significant correlation between IL-7 levels and birth weight was noted. It is possible that the anti-retroviral drugs are autonomously regulating the placental cytokine production as well as tit anti-HIV effect (Faye *et al*., 2007). Interestingly, a study has linked antiretroviral therapy to lower birth weight infants regardless of gestational age, which may be linked to both maternal and fetal TNF-α production (Kasahara *et al*., 2013). In contrast, more recent studies indicate that ART reportage either as ZDV prophylaxis or triple ARV schedules, is correlated with lower probabilities for adverse birth outcomes (Moodley *et al*., 2016). However, there is greater likelihood for poor birth outcomes amongst HIV infected women who are unregistered for antenatal care and therefore untreated (Moodley *et al*., 2016). However, it is possible that factors such as socio-economic status, the initiation of ART either during pregnancy or before pregnancy as well as ART duration prior to delivery may also influence the birth outcomes (Moodley *et al*., 2016).

Christian and Porter (2014) also demonstrated elevations in both IL-6 and TNF-α throughout pregnancy and postpartum, whilst IL-8 and IL-1b declined during the early gestational period and later into pregnancy, but heightened at postpartum (Christian & Porter, 2014). Previous studies demonstrated higher Th-2 cytokine levels and a greater immunity during the course of pregnancy in the HIV-uninfected groups in contrast to the HIV-infected groups (Kolte *et al*., 2011). Moreover, increased IL-4 and IL-10 concentrations were observed in HIV-ve women in comparison to HIV+ve women (Kolte *et al*., 2011). It is possible that these increased cytokine concentrations are hormone induced since progesterone promotes the production of IL-4 and IL-5 (M-P Piccinni *et al*., 2000). However, we only report cytokine concentrations measured at one gestational point, nonetheless, our Pearson's correlation analyses demonstrate an inverse relationship between IL-1b, Il-8 and IL-4 with gestational age. Our findings corroborate that reported by Christian and co-workers, who demonstrated a U-shaped curve for IL-8 and IL-1b, suggestive of cytokine reductions during mid to late gestation in comparison to early pregnancy (Christian & Porter, 2014). This is indicative of the foundational inflammatory response observed in early pregnancy which down-regulates over time (Christian & Porter, 2014). However, our data failed to show any visible differences in the concentrations of IL-8 and IL-1b when compared between HIV status and antiretroviral treatment status.

Interestingly, we also demonstrate a significant correlation between TNF-alpha levels and gestational age, similar to that reported by Christian and Porter (2014). More recently, antiretroviral therapy correlates with a major reduction in the levels of IL-2, TNF-α and IL-6 in pregnancy (Maharaj *et al*., 2017). Despite the lack of statistical significance, our data demonstrate similar reductions for Il-6 and TNF-α amongst those on ART compared to those that aren't. Tumor necrosis factor alpha (TNF-α) is linked to the

cell mediated cytotoxic arm of the specific immune response whilst the inflammatory IL-2 controls both the cellular and humoral immune reaction (Fiore *et al*., 2006; Osakwe *et al*., 2010). We also reveal an inverse relationship between IL-2 and gestational age, albeit non-significant. Our data corroborates the reduction in IL-2 through pregnancy, as observed by Russell *et al*. (1997) (Russell *et al*., 1997). Likewise, Shimaoka *et al*., (2000) also revealed reductions in the concentrations of IL-2, IFN-g, IL-4 and IL-10 throughout pregnancy (Shimaoka *et al*., 2000). These cytokines represent the Th1 and Th2 immune response, indicative that an overall reduction in maternal immunity manifests during pregnancy.

Despite the lack of statistically significant difference, our data report the potential for the onset of anemia in the HIV-ve pregnant group. It is possible that the levels of hemoglobin may be associated with the increase in plasma volume during pregnancy. We also demonstrate a noticeable difference in hemoglobin levels between those receiving treatment in contrast to those not on treatment amongst the HIV positive group. The hemoglobin levels were higher amongst those receiving treatment, indicative of the possibility of being anemic. Our data also supports Nandlall and co-workers, who report higher predisposition to anemia development amongst HIV+ve pregnant women on antiretroviral therapy (Nandlal *et al*., 2014).

Despite the lack of a statistical significance, we demonstrate a trend in IL-17A expression between the HIV+ve and -ve cohorts, as well as treated versus untreated HIV participants. The HAART treated women had higher cytokine expressions compared to untreated women. Interleukin 17-A is pro-inflammatory in nature and exerts its effect in response to activation of the inflammatory effectors, the antimicrobial molecules defensins and mucins, the chemokines, the inflammatory IL-6 and tumor necrosis factor-α, and the anti-inflammatory granulocyte colony-stimulating factor (Onishi & Gaffen, 2010). It is possible that the restorative immune effect of ART is associated with this pro-inflammatory effect. Moreover, the proinflammatory role of IL-17A in pre-eclampsia development received authentication, based on elevated levels observed in pre-eclamptic pregnancies compared with normotensive pregnancies (Molvarec *et al*., 2015). However, a limitation of our study is that the circulating values of several cytokines in our study were below the detection limit of the assay. This may be attributed to the use of the Multiplex bead array technology, indicative that this technology may not be the ideal platform for measuring analytes with very low concentrations due to it elevated dynamic range.

In conclusion, this study demonstrates midgestational variation in serum inflammatory and anti-inflammatory immunologic profile of pregnant women, regardless of the use of antiretroviral therapy. This disparity in the vulnerable HIV infected women will affect progression of pregnancy and promote fetal morbidity and mortality.

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