

Full-Length Research Article

# Does HAART Dysregulate Angiogenesis in HIV-infected Pre-eclampsia?

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**Summary:** A dysregulation of angiogenic mediators has been implicated in HIV infection. Inconsistent data exists on highly active antiretroviral therapy (HAART) usage in pregnancy and its association with PE development. In view of the high prevalence of HIV infection and PE in SA, this study was aimed at determining PlGF and sFlt-1 levels in HIV-infected normotensive and preeclamptic pregnancies treated with HAART. Both PlGF and sFlt-1 were quantified in serum from HIV positive [normotensive (N+) and preeclamptic (P+)] and HIV negative [normotensive (N-) and preeclamptic (P-)] pregnancies, using a Milliplex Multiplex immunoassay. sFlt-1 was significantly upregulated in P+ vs the N+ groups. PlGF was significantly downregulated in PE vs normotensive groups, regardless of HIV status. sFlt-1/PlGF ratio was significantly increased in PE- vs the N- groups. We report an amplification of sFlt-1 in lieu of PlGF down-regulation in HIV-infected pregnancies receiving HAART.

**Keywords:** sFlt-1, PlGF, angiogenic dysregulation, HIV-1, HAART

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## INTRODUCTION

In low-to-middle-income countries, maternal mortality is a public health challenge due to late initiation of and irregular visits for antenatal care. Most of these deaths are associated with hypertensive disorders of pregnancy (HDP), more specifically preeclampsia (PE) (Peres *et al.*, 2018). In some parts of Africa, 18% of pregnancies are complicated by PE (Robertson, 2019). In South Africa (SA), the overall HIV prevalence rate is 13.7% (8.2 million), of which 25% are women in their reproductive age (Stats SA, 2021). It is disturbing that antenatal surveillance in the province of KwaZulu-Natal (KZN), SA revealed that 40% of pregnant women are HIV positive (Woldesenbet *et al.*, 2018). Whilst the administration of antiretroviral therapy (ART) prevents mother-to-child transmission of HIV (Clouse *et al.*, 2020) it promotes PE development due to immune reconstitution (Phoswa *et al.*, 2019, Landi *et al.*, 2014).

The multifactorial disease of PE presents itself in the form of hypertension in the latter half of pregnancy (>20 gestational weeks) with/without proteinuria and multi-organ dysfunction (Brown, 2018). In KZN, the prevalence of PE is 12% (Moodley *et al.*, 2016). Currently, the only effective cure for PE is emergency delivery of the fetus and placenta. The underlying pathogenesis of PE is associated with inadequate placentation due to deficient trophoblast invasion and absence of myometrial spiral artery remodelling (Fisher, 2015). Consequently, this state of under-perfusion creates placental hypoxia with localized oxidative stress (Valenzuela *et al.*, 2012).

PE is an anti-angiogenic state as a result of increased release of anti-angiogenic factors into the maternal circulation. In PE, soluble fms-like tyrosine kinase-1 (sFlt-1) antagonizes the biological availability of both vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) by binding to their receptors (Maynard and Karumanchi, 2011). This angiogenic imbalance contributes to widespread maternal endothelial injury and heightened inflammatory response with clinical manifestation of PE (Chaiworapongsa *et al.*, 2009). Moreover, sFlt-1 via VEGF inhibition stimulates complement-mediated placental injury in PE (Yonekura Collier *et al.*, 2019). A dysregulation of angiogenic mediators have also been implicated in HIV infection (Chaiworapongsa *et al.*, 2009). Conflicting data exists on ART (HAART) usage in pregnancy and its association with PE development (Powis *et al.*, 2013).

Investigation of angiogenic factors in PE has been evaluated for its use in the early prediction and assessment of disease severity and progression (Verlohren *et al.*, 2010, Zeisler *et al.*, 2016). Given the high prevalence of HIV infection and PE in SA, this study aimed to determine PlGF and sFlt-1 levels in HIV infected normotensive and preeclamptic pregnancies treated with HAART.

## MATERIALS AND METHODS

**Study population:** Following receipt of ethical approval (BE313/18), regulatory health permissions and written informed consent, venous blood samples were collected from 80 pregnant women (n=80) attending an antenatal

clinic at a large regional hospital in eThekweni, KZN. Serum samples were stored at -80°C until immunoassay. The study population (n=80) consisted of normotensive pregnant (n=40) and preeclamptic (n=40) groups, which were further stratified by HIV status [*i.e.*, HIV positive normotensive (N<sup>+</sup>; n=20); HIV negative normotensive (N<sup>-</sup>; n=20); HIV positive preeclamptic (P<sup>+</sup>, n=20) and HIV negative preeclamptic (P<sup>-</sup>, n=20)]. Preeclampsia was defined as systolic blood pressure  $\geq$  140 mmHg and diastolic blood pressure  $\geq$  90 mmHg with at least a two plus of proteinuria on dipstick testing after 20 weeks of pregnancy (Brown, 2018).

The relevant data of all research participants were obtained from their maternity case records. HIV testing was done after counselling using a rapid point-of-care test kit initially, as is the standard of care in South Africa. All patients recruited were of African ancestry and resident in the same geographical location in order to maintain ethnographic and anthropometric consistency. All newly diagnosed HIV-positive pregnant women were initiated on combined anti-retroviral therapy (cART) consisting of tenofovir, emtricitabine, and efavirenz, as per South African national HIV guidelines at the time of the study (South African National Department Of Health, 2020). Exclusion criteria was based on patients that declined participation and those with polycystic ovarian syndrome, abruption placentae, intrauterine death, sickle cell disease, chronic renal disease, cardiac disease, unknown HIV status, pre-existing seizure disorders and asthma.

**Milliplex Multiplex assays:** Serum was diluted for quantifying PIGF (1:3) and sFlt-1 (1:5) prior to quantification using MILLIPLEX®MAP Human Angiogenesis/Growth Factor Magnetic Bead Panel 1 (Cat.# HAGP1MAG-12K) and MILLIPLEX®MAP Human Angiogenesis Magnetic Bead Panel 2 (Cat.# HANG2MAG-12K) kits respectively. The immunoassays were performed according to the manufacturer's instructions with an overnight incubation (16-20hrs) at 2-8°C for both PIGF and sFlt-1. The plates were then analysed with the Bio-Plex MAGPIX Multiplex reader (Bio-Rad Laboratories, Pleasanton, CA, USA) with xPONENT v.3.2 software.

## Statistical analysis

All data was analysed using STATA (version 12, STATAcorp) and Graph Pad Prism version 8 (California, USA). Data were tested for normality using the D'Agostino test and histograms are presented as median and interquartile range (IQR). The Kruskal Wallis test and the Dunn's *post hoc* test were used to determine if there was a significant difference between groups. The Spearman's correlation coefficients were used to assess the relationship between sFlt-1 and PIGF between groups and between clinical factors. A *p* value  $\leq$  0.05 was considered statistically significant at a 95% confidence level.

## RESULTS

**Clinical demographics:** The clinical characteristics are shown in Table 1. As expected, the systolic (SBP) and diastolic (DBP) blood pressures were significantly higher in PE compared to normotensive pregnancies ( $p < 0.0001$ ). Gestational age at delivery were significantly lower in PE to relative to normal pregnancies ( $p < 0.0001$ ). There was a lack of statistically significant difference for maternal age and maternal weight between normotensive pregnant and PE women, regardless of pregnancy type or HIV status.

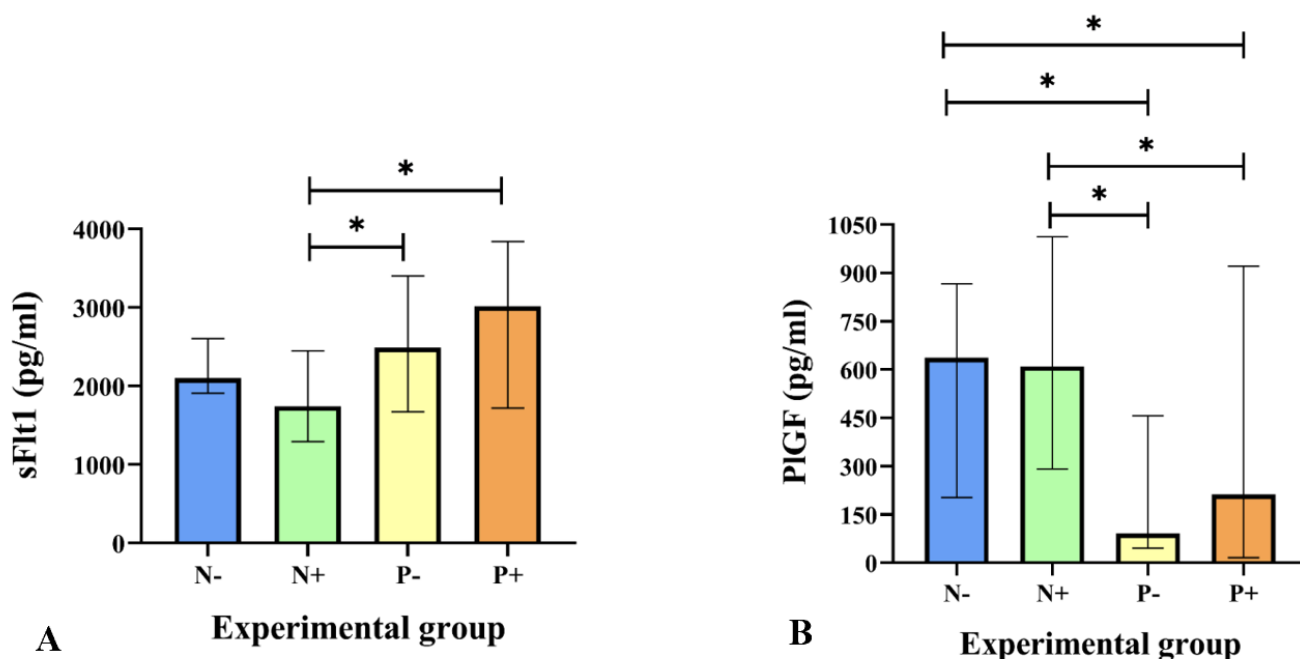
**Serum levels of sFlt-1 and PIGF:** The serum levels of sFlt-1 and PIGF are shown (Figure 1A-B). The Kruskal Wallis and Dun's *post hoc* test revealed a statistically significant difference for circulating levels of sFlt-1 between the P- vs the N+ groups, as well as between the P+ vs the N+ groups (Figure 1A). Likewise, a statistically significant difference was noted for PIGF levels between the P- versus N-; and P- vs N+ groups ( $p = 0.0001$ ). Moreover, we noted a significant difference between P+ vs the N-, and P+ vs the N+ groups ( $p = 0.0001$ ) respectively (Figure 1B). For the sFlt-1/PIGF ratio, a statistically significant difference was noted between the P- vs N-, and P- vs the N+ groups ( $p = 0.04$ ), respectively.

**Table 1.**

Clinical characteristics across all study groups [N=80; Median (25th – 75th percentile)]

	Normotensive (n=40)		Pre-eclamptic (n=40)		<i>p</i> value
	HIV negative (N-) (n=20)	HIV positive (N+) (n=20)	HIV negative (P-) (n=20)	HIV positive (P+) (n=20)	
Maternal age (years)	26.00 (19.00-38.00)	28.00 (23.00-33.00)	25.00 (21.00-32.00)	29.00 (26.00-30.00)	0.962
Maternal weight (kg)	75.00 (67.00-85.00)	70.35 (65.10-75.50)	75.50 (68.00-103.00)	70.00 (67.00-81.00)	0.281
Gestational age (wks)	39.00 (39.00-39.00)*	38.00 (38.00-40.00)*	35.00 (34.00-38.00)*	34.00 (34.00-36.00)*	<b>0.0001*</b>
Systolic bp (mmHg)	120.00 (110.00-125.00) *	118.50 (114.50-126.50) *	168.00 (160.00- 177.00)*	170.00 (156.00-178.00) *	<b>0.0001*</b>
Diastolic bp (mmHg)	69.00 (57-73)*	73.00 (65.00-81.00)*	105.50 (98.00-112.00)*	106.00 (97.00-114.00)*	<b>0.0001*</b>

\* $p < 0.05$  considered statistically significant

**Figure 1:**

Serum concentrations (A–B, median and interquartile range) of (A) anti-angiogenic sFlt-1 (pg/ml) and (B) angiogenic PIGF (pg/ml) in HIV negative normotensive pregnant (N-); HIV positive pregnant (N+); HIV negative preeclamptic (P-) and HIV positive preeclamptic (P+) women.

**Spearman's correlation matrix between circulating levels of sFlt-1 and PIGF:** A bivariate Spearman's correlation analysis showed a relationship between circulating sFlt-1 and PIGF levels with clinical factors, in the N+ and P+ groups. A negative association between SBP and gestational age ( $r=-0.68$ ;  $p=0.003$ ); and a positive association between DBP and SBP ( $r=0.65$ ;  $p=0.006$ ) was noted.

Based on HIV status and pregnancy type, a statistically significant negative association was observed between sFlt-1 (P-) group and DBP ( $r=-0.57$ ,  $p=0.002$ ); and between sFlt-1 (P-) groups and sFlt-1 (P+) groups ( $r=-0.58$ ,  $p=0.01$ ). For PIGF, a statistically significant negative association was observed between P- group and SBP ( $r=-0.64$ ,  $p=0.04$ ). A statistically significant positive association was observed between sFlt-1 (P-) and PIGF (N-) groups ( $r=0.79$ ;  $p=0.02$ ). However, based only on HIV status and regardless of pregnancy type, a statistically significant negative association was shown between SBP and gestational age ( $r=-0.86$ ,  $p=0.0001$ ); DBP and gestational age ( $r=-0.74$ ;  $p=0.000$ ) and DBP and SBP ( $r=0.92$ ,  $p=0.000$ ) respectively. We also noted a positive association between sFlt-1/PIGF ratio and SBP ( $r=0.40$ ;  $p=0.03$ ); and DBP ( $r=0.4$ ;  $p=0.03$ ), in contrast to the negative association observed between the sFlt-1/PIGF ratio and gestational age ( $r=-0.50$ ;  $p=0.006$ ). A similar correlation analyses were done based on pregnancy type (normotensive vs PE); however, no significant associations were observed between the analytes and the clinical factors

## DISCUSSION

In our study, we report a statistically significant up-regulation of sFlt-1 in P+ compared to the N+ groups ( $p<0.05$ ), albeit similar levels were observed between N- vs the P- groups. It is widely accepted that sFlt-1 augmentation causes peripheral vasoconstriction with development of high blood pressure in PE, however, the altered sFlt-1 levels in our study may also be amplified from ART usage. In SA, it is a standard practice for pregnant women with previous antiretroviral exposure and documented viral load suppression, to be administered a fixed dose combinations of Tenofovir (TDF) 300mg; Lamivudine (3TC) 300mg and Dolutegravir (DTG) 50mg, loosely labelled (TLD) (Nel *et al.*, 2020). Women in our study did not switch to the revised guidelines that include DTG. Instead, these women remained on Tenofovir (TDF) + Lamivudine (3TC)/Emtricitabine (FTC) + Efavirenz (EFV) to continue their ART regimen.

In contrast to HIV-protease inhibitors (PIs), the nucleoside reverse transcriptase inhibitors (NRTIs) (*viz.*, zidovudine, efavirenz, lamivudine, emtricitabine, abacavir or tenofovir) induces endothelial dysfunction via increased reactive oxygen species production, and defective mitochondrial activity (Chen *et al.*, 2019). Notably, NRTIs interferes with angiogenic signal transduction (Weiß *et al.*, 2016, Song *et al.*, 2018, Chen *et al.*, 2019), including the mitogen-activated protein kinases (MAPK)/extracellular-signal-regulated kinases (ERK) and/or the phosphoinositide 3 kinase (PI3K)/ protein kinase B (AKT) pathways (Loizzi *et al.*, 2017). Furthermore, NRTIs suppress receptor tyrosine kinase (RTK) signalling by prohibiting VEGF-A/PIGF induced endothelial cell neovascularization, suggesting a direct effect of NRTIs on RTK activation (Song *et al.*, 2018). The dysfunctional angiogenic expression in our study

may be a consequence of NRTI suppression on VEGFR-2 activity and the inactivation of the MAPK/ERK pathway, which prevents endothelial proliferation (Liang *et al.*, 2018). Moreover, previous studies have reported that preeclamptic females receiving HAART are associated with endothelial dysfunction and angiogenic imbalance (Ajadi *et al.*, 2021). Our findings also corroborate previously reported data on HIV uninfected pregnant women (Hirashima *et al.*, 2005, Maynard *et al.*, 2003, Levine *et al.*, 2004).

An anti-angiogenic phenotype was reported between second and third trimester pregnancies (Romero *et al.*, 2010), similar to our findings albeit at term. Earlier studies establishing the reference range of sFlt-1 suggest that sFlt-1 increases with gestational age (Levine *et al.*, 2004, Thadhani *et al.*, 2004), however, we show no significant association between sFlt-1 and gestational age. Off note, an anti-angiogenic state is associated with adverse birth outcomes in HIV infected women (Conroy *et al.*, 2017).

In our study, similar levels of sFlt-1 expression were noted between the HIV infected vs uninfected groups. In a hypoxic environment, mitochondrial reactive oxygen species (mtROS) trigger the release of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ). An increased production of ROS has been previously reported in HIV-1 infection due to CD4<sup>+</sup> T cell apoptosis (Perl and Banki, 2000). HIF-1 $\alpha$  then promotes viral replication of HIV-1 in CD4<sup>+</sup> T cells and stimulates the release of extracellular vesicles (EVs) from infected cells (Duette *et al.*, 2018). HIV-1 infection thereby enhances HIF-1 $\alpha$  transcriptional activity. Duette *et al.* (2018) suggests that cART with undetected viral load may stimulate the activity of HIF-1 $\alpha$  with resultant inflammation (Duette *et al.*, 2018). It is also possible that the hyper-reactivity of HIF-1 $\alpha$  irrespective of ART promoted *Flt-1* gene activity, amplifying sFlt-1 expression in our study.

As expected, we noted a significant down-regulation in PIGF expression in the PE groups compared to the normotensive groups, regardless of HIV status. Under conditions of pathology, a permanent angiogenic imbalance exists in contrast to transient activation of physiological angiogenesis (Voron *et al.*, 2014). In turn, VEGF and PIGF exhibit immunomodulatory properties by inducing an immunosuppressive environment (Voron *et al.*, 2014, Dikov *et al.*, 2005). Therefore, the down-regulation of PIGF in PE in our study may originate from the loss of maternal tolerance induced by HIV infection together with inadequate trophoblast invasion and deficient vascular development (Albonici *et al.*, 2020). Additionally, the decreased expression of PIGF in the HIV-positive groups regardless of pregnancy type, may be attributed to PIGF/TNF- $\alpha$  stimulation. HAART is known to modify systemic TNF- $\alpha$  induced inflammation (Keating *et al.*, 2011). Notably, a shift from Th2 to Th1 immunity has been described as one of the confounding effects of HAART (Lopez *et al.*, 2012). While HAART prevents HIV disease progression, its usage is associated with adverse pregnancy outcome since normal pregnancy favours a Th2 response (Lin *et al.*, 2007). High PIGF levels produced by the placenta supports a Th2 cytokine profile in favour of normal fetal development (Lin *et al.*, 2007). In our study, the excess sFlt-1 antagonizes PIGF activity by acting as a decoy receptor; however, their levels are exacerbated by the immune restorative action of ARVs.

We report a significant up-regulation of sFlt-1/PIGF

ratio in the PE- versus the N- groups. In HIV uninfected samples, sFlt-1 binds to circulating VEGF and PIGF, inhibiting the action of these transmembrane receptors. Due to the abnormal vascular remodelling of the maternal spiral arteries, the resultant hypoxic environment promotes the excessive release of sFlt-1 into the maternal circulation (Lecarpentier and Tsatsaris, 2016). Preeclampsia arises when the functional activity of sFlt-1 surpasses that of VEGF (Rana *et al.*, 2007). Notably, a high sFlt-1/PIGF ratio is associated with an increased risk of PE development (Zeisler *et al.*, 2016).

HIV accessory proteins including *Tat*, *gp120* and *p17* contribute to endothelial vascular injury in HIV-1 infection (Jiang *et al.*, 2010, Wang *et al.*, 2014). The *Tat* protein mimics VEGF due to its arginine and lysine-rich sequence similarities to VEGF and targets its VEGFR-2/KDR receptor; hence it is a powerful angiogenic protein (Albini *et al.*, 1996, Zhou *et al.*, 2013). Furthermore, *Tat* gains access to the endothelium to facilitate HIV-1 induced oxidative stress through the arginine-glycine-aspartate motif; via the integrins  $\alpha_v\beta_3$  and  $\alpha_5\beta_1$  and VEGFR-2/KDR of its basic domain. It negatively regulates endothelial cell morphology, gene expression, and proliferation via aberrant cell signalling and MAPK activation (Kline and Sutliff, 2008). Interestingly, ART does not inhibit *Tat* secretion (Mediouni *et al.*, 2012). Additionally, the HIV-1 glycoprotein *gp120* prompts the release of *Tat* by binding to heparin sulphate proteoglycans on endothelial cells where it induces oxidative stress (Crublet *et al.*, 2008). Consequentially, endothelin-1 is released and induces vasoconstriction (Anand *et al.*, 2018). The HIV-1 matrix protein *p17* persists in lymph nodes of patients receiving HAART (Popovic *et al.*, 2005, Fiorentini *et al.*, 2006), deregulating the biological activity of immune cells (Caccuri *et al.*, 2012), thereby affecting angiogenesis.

In conclusion, this study demonstrates an anti-angiogenic (sFlt-1 vs PIGF) milieu in PE. We report sFlt-1 amplification in lieu of PIGF downregulation in HIV infected pregnancies. Moreover, the HIV-1 *Tat*, *gp120* and *p17* proteins are responsible for endothelial injury contributing to angiogenic imbalance. The endothelial dysfunction may be a direct effect of these HIV proteins or from indirectly via HAART administration. Both PIs and NRTIs dysregulate angiogenesis via RTK cell signalling pathways. Further large-scale investigations are warranted to fully ascertain the role of HAART on angiogenesis during pregnancy.

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