

Circulating soluble fms-like tyrosine kinase-1, soluble endoglin and placental growth factor during pregnancy in normotensive women in KwaZulu-Natal, South Africa

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Abstract

Background: Based on the increased pre-eclampsia and HIV antenatal incidence in South Africa, we determined the angiogenic profiles due to its mechanistic link in preeclampsia development, throughout uncomplicated pregnancies in HIV positive and negative women.

Objective: To determine the angiogenic profiles throughout uncomplicated pregnancies in HIV positive and HIV negative women. We explored possible correlations between angiogenic serum levels and selected maternal characteristics (HIV status, gestational age, maternal factors, and pregnancy outcomes).

Method: This study was conducted at a primary health care facility in Durban, South Africa. Forty-six pregnant women aged 18-45 years, were enrolled at 10-20, 22-30 and 32-38 weeks' gestation, respectively through convenient sampling. Serum samples were collected and quantitatively evaluated using ELISAs. Clinical and epidemiological data were analysed using STATA (version 14). A probability level of $p < 0.05$ was considered statistically significant.

Results: Of those enrolled, 28.3% were nulliparous, 82% were HIV positive and none developed pre-eclampsia. Systolic and diastolic blood pressure increased slightly throughout pregnancy. Fluctuating angiogenic and anti-angiogenic levels were demonstrated during pregnancy.

Conclusion: This study contributes to the current angiogenic knowledge in normotensive pregnancies, and may assist as a reference range against which these factors may be compared in HIV complicated pregnancies.

Keywords: sFlt-1, PlGF, sEng, pregnancy, HIV.

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Introduction

Despite the significant decrease in maternal morbidity and mortality worldwide, the WHO 2015 estimates highlight that approximately 303,000 maternal deaths will occur by 2015, with a maternal mortality rate of 216 per 100,000 live births during pregnancy, at delivery or postnatally, most cases occurring in low- and middle-income coun-

tries¹. Suboptimal antenatal care in South Africa is linked with poorer uptake of Human Immunodeficiency Virus (HIV) testing and adverse maternal and birth outcomes^{2,3}. In developing and resource constrained countries such as South Africa, HIV is responsible for an estimated 40% of all maternal deaths⁴. The antenatal HIV prevalence of approximately 40% is the highest in KwaZulu-Natal, South Africa⁵. To date, the most effective intervention used to prevent mother-to-child-transmission is the use of highly active antiretroviral treatment (HAART)⁴, which alters maternal immunological responses thus improving maternal and perinatal outcomes^{4,6}. Despite the obvious benefits of HAART in pregnant women, the rates of adverse birth outcomes is still reportedly higher in HIV positive women on HAART than HIV-uninfected women^{6,7}. Moreover, earlier studies have shown inconsistencies in the angiogenic expression in women on anti retrovirals in comparison to HIV uninfected women^{8,9}, whilst a more recent South African study suggest that HIV possibly jeopardizes vascular perfusion during pregnancy, particularly placental development¹⁰.

Fetal development relies on adequate placental growth which is dependent on branching and non branching angiogenesis^{11,12}. Branching angiogenesis occurs during the first 24 weeks of pregnancy, under relative hypoxic conditions, in contrast to third trimester non branching angiogenesis¹². Placental trophoblasts secrete angiogenic placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) into the maternal vasculature, supporting endothelial proliferation and survival, and arterial remodeling^{13,14}. The bioavailability of both VEGF and PlGF is controlled by the anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1), which is a splice variant of the VEGF receptor^{15,16}. Likewise, increased circulating levels of soluble endoglin (sEng) was also demonstrated and implicated in PE development¹⁷. Moreover, this placental derived protein competes with endoglin to bind to circulating transforming growth factor beta (TGF- β), thus weakening its signaling¹⁷. Walshe et al.¹⁸ suggests that the impaired signaling of VEGF and/or TGF- β pathways may contribute to the endothelial dysfunction that characterizes PE development. This disrupted angiogenic expression is linked with fetal growth restriction¹⁹, preterm delivery²⁰ as well as hypertensive disorders of

pregnancy (HDP), including preeclampsia (PE)²¹, coronary artery disease²², heart failure and maternal vascular malperfusion¹⁰. It is possible that the altered placental perfusion contributes systemic alterations in the circulating expression of angiogenic factors²³.

Pregnancy induced hypertensive disorders like PE are believed to occur in response to an immune-induced placental alteration evoked possibly by HIV and HAART mediated immune disturbances¹⁰. PE is characterized by increased circulating sFlt-1 levels, which inhibit angiogenic signaling and subsequently induce endothelial dysfunction²⁴. Placental delivery is no longer an option for PE resolution since epidemiological evidence confirms the onset of various long-term cardiovascular disorders, long after the dissolution of preeclampsia symptoms^{21,25}. Despite the improved understanding of PE, delayed child-bearing combined with maternal body mass index (BMI), maternal age, and parity, may be correlated with angiogenic balance even in uncomplicated pregnancies^{26,27}.

Earlier studies demonstrates the clinical role of maternal PlGF and sFlt-1 levels as valuable predictors of preeclampsia development and its associated consequences in both mother and fetus^{28,29}, thereby supporting their increased use in both maternal and fetal investigations because of its link with placental development³⁰. Others have also recommended that evaluating maternal concentrations of both sFlt-1 and sEng may be helpful in the prognosis of pregnancy complications such as preeclampsia and intrauterine growth restriction^{19,31,32}. Whilst we together with several others, have evaluated the angiogenic profiles in normal pregnancies as a means of comparing them with complicated pregnancies, the intention of such data was not intended at instituting reference values^{28,33,34}.

We therefore aimed to determine the angiogenic and anti-angiogenic profiles through three gestational periods from uncomplicated pregnancies in HIV positive (HIV +ve) and HIV negative (HIV -ve) women. We further explored possible correlations between serum levels of these analytes and selected maternal characteristics (HIV status, gestational age, maternal factors, and pregnancy outcomes). Since there was limited South African data,

we intended to contribute to the current knowledge on serum levels of sFlt-1, PlGF and soluble endoglin (sEng).

Methods

This was a prospective study which was nested within a birth cohort study. It was conducted at a primary health care (PHC) facility in Durban, South Africa between 2015 and 2016. Antenatal care in South Africa is usually initiated before 20 weeks of gestation as prescribed by the National Department of Health³⁵. Only participants who presented for their first antenatal visit, prior to 20 weeks gestation and who consented to blood collection at the 3 gestational periods were included in the study, which resulted in a final convenient sample number of 46. Participants could not be followed until delivery, since PHC facilities feed into various public health hospitals, resulting in logistical challenges in retaining all participants until delivery. Adolescent pregnancies (<18 yrs), women presenting with chronic diabetes, chronic hypertension, gestational diabetes, connective tissue disorder, cardiac disease, sickle cell disease, anti-phospholipid antibody syndrome, chorioamnionitis, unknown HIV status, and those unable to provide informed consent were excluded. Following institutional (Durban University of Technology Institutional Research Ethics Committee, IREC 045/14) and department of health (HRKM 234/14) permission, written and informed consent was obtained from all participants recruited through convenient sampling. Blood samples were collected between 10-20 weeks, 22-30 weeks and 32-38 weeks via venipuncture in serum separator tubes from pregnant women aged 18-45 years, every time blood pressure was measured. Clinical and demographic variables including maternal age, weight and blood pressure, BMI, parity, HIV status, maternal hemoglobin (Hb)

levels and birth weight, were derived from chart registers and administered epidemiological questionnaires respectively. HIV status was confirmed at the first antenatal visit and if HIV positive, were immediately placed on antiretroviral therapy for prevention of mother to child transmission. Blood samples were centrifuged at 3500 rpm, 4°C for 5 min and serum samples were aliquoted and stored at -80°C until analysis. Serum concentrations of sFlt-1 (1:5), sEng (1:5) and PlGF (1:2) were measured in triplicate by ELISA according to the manufacturer's protocol (R&D Systems, Minneapolis, MN).

Statistical analysis

All statistical analyses were conducted using STATA (version 14). Data were assessed for their distribution by the Shapiro–Wilk test. The homogeneity of variance between groups was assessed by Levene's test for equality of error variances. Descriptive statistics was utilized and outcome variables are presented as means and median (interquartile range) depending on data distribution. Parity was a dichotomous variable evaluated as nulliparous or multiparous. BMI was evaluated both as a continuous and categorical variable. BMI was dichotomized into ≤ 24.99 kg/m² (normal) and >25 kg/m² (overweight). The Friedman's ANOVA test was used to compare the levels of biomarkers stratified by parity, BMI and HIV status. The Spearman's rank correlation test was computed to assess the relationship between serum levels of PlGF, sFlt-1 and sEng and maternal factors. A probability level of $p < 0.05$ was considered statistically significant.

Results

A total of 46 participants were followed at 10-20, 22-30 and 32-38 weeks' gestation, respectively. Demographic and clinical characteristics are presented in Table 1.

Table 1: Clinical characteristics of maternal cohort with normotensive pregnancies (n=4

Maternal Characteristics	
Maternal age at delivery (yrs) (Mean, SD)	25.76 (5.01)
Smoking (n, %)	2 (4.35)
Alcohol use (n, %)	4 (8.70)
Height (cm)(mean, SD)	1.59 (0.57)
Weight (kg) (median, IQR)	
10-20 weeks	62.60 (60.00-71.00)
22-30 weeks	68.15 (63.00-74.30)
32-38 weeks	72 (66.40-80.00)
Body Mass Index (BMI, kg/m²) (median, IQR)	
10-20 weeks	25.27 (22.74-28.53)
22-30 weeks	27.15 (23.95-30.52)
32-38 weeks	28.35 (25.85-32.46)
Blood pressure (mmHg)(mean, SD)	
<i>Systolic</i>	
10-20 weeks	101.50 (11.05)
22-30 weeks	108 (10.21)
32-38 weeks	110 (10.30)
<i>Diastolic</i>	
10-20 weeks	60 (8.12)
22-30 weeks	68 (9.22)
32-38 weeks	70 (14.32)
Parity (n, %)	
Nulliparous	13 (28.26)
Primiparous/multiparous	33 (71.74)
HIV Status (n, %)	
Positive	38 (82.61)
Negative	8 (17.39)
Birth Weight (kg) (mean, SD)	3.01 (0.40)

The mean maternal age at delivery was 25 years, whilst the mean birth weight was 3.01 kg. Of the total sample, 28% were nulliparous and 82% were HIV positive. Both systolic and diastolic blood pressure increased slightly throughout the defined gestational intervals. Median circulating levels of PIGF, sFlt-1 and sEng throughout

pregnancy are presented in Table 2. Both PIGF and sFlt-1 levels increased throughout pregnancy, while soluble endoglin dropped around 30 weeks' gestation and thereafter stabilized to its initial levels. In contrast, both the indices of vascular disturbance (sFlt-1/PIGF and sFlt-1+sEng/PIGF), decreased considerably towards the end of 2nd and 3rd trimesters.

Table 2: Serum levels of PIGF, sFlt-1, sEng and antiangiogenic ratios sFlt-1/PIGF; (sFlt-1 + sEng)/PIGF (Median, IQR, n = 46)

Gestational period (wks)	Angiogenic factor	Antiangiogenic factor		Antiangiogenic ratios	
	PIGF pg/mL	sFlt1 pg/mL	sEng (ng/ml)	sFlt1/PIGF	(sFlt1 + sEng)/PIGF
10-20 wks	301.52 (201.71)	1169.17 (825.12)	5.08 (1.99)	5.23 (11.3)	22.31(35.02)
22-30 wks	642.48 (374.27)	1244.17 (936.42)	3.94 (1.65)	2.20 (2.24)	8.20 (7.42)
32-38 wks	713.53 (534.28)	1358.07 (870.87)	5.33 (3.06)	1.81 (2.71)	7.55 (5.89)

Median serum levels of PIGF, sFlt-1 and sEng throughout pregnancy, stratified by parity and BMI are shown in Table 3. There were no statistically significant differences noted based on this stratification. Median PIGF levels in multiparous women increased progressively throughout pregnancy however, in nulliparous women, there was an increase in serum PIGF levels between 10-30 weeks with a reduction of 130 pg/ml at 32 weeks' gestation (Table 3). In contrast, sFlt-1 levels dropped between 22-30 weeks in nulliparous women compared to multiparous and thereafter increased. Likewise, a reduction in sEng

levels at 22-30 weeks' gestation was noted amongst the nulliparous and multiparous women, which subsequently increased at 32 week's (Table 3). With regard to the effect of BMI on the angiogenic profiles, PIGF levels increased progressively throughout pregnancy, irrespective of BMI < 24.99 or > 25.00 kg/m². The concentration of sFlt-1 increased amongst those who had a BMI < 24.99 kg/m² in contrast to those > 25.00 kg/m². Levels of sEng however declined between 22-30 weeks and thereafter increased at 32 week's irrespective of BMI < 24.99 or > 25.00 kg/m² (Table 3). There were no statistically significant differences observed based on this stratification.

Table 3: Effect of parity and BMI on angiogenic and antiangiogenic factors stratified by gestational periods in normotensive pregnancies (n=46, median, IQR)

Angiogenic Profiles	Parity		BMI	
	Nulliparous	Multiparous	≤ 24.9	≥ 25
PIGF (10 -20 wks)	244.08 (81.14-340.59)	307.58 (143.89-398.16)	316.28(161.10-371.88)	236.73(139.11-326.25)
PIGF (22-30 wks)	757.13 (483.53-886.61)	569.02 (420.38-798.07)	752.81(486.53-862.19)	480.85 (350.63-771.40)
PIGF (32-38 wks)	627.04 (444.79-1059.38)	713.53 (247.80-854.24)	808.05 (483.86-956.55)	483.86 (200.58-784.72)
sFlt-1 (10 -20 wks)	1457.98 (955.80-1671.09)	956.87 (582.75-1422.34)	1033.15 (779.87-1705.77)	1335.08 (625.08-1457.98)
sFlt-1 (22 -30 wks)	1244.14(939.25-1748.67)	1260.31 (790.72-1724.39)	1384.33 (882.82-1778.57)	1228.58(746.33-1642.00)
sFlt-1 (32- 38 wks)	1594.77 (971.57-1996.57)	1210.92 (750.61-1594.03)	1427.05 (1056.63-1779.14)	1093.91 (606.50-1594.77)
sEng (10 -20 wks)	5.08 (3.97-5.76)	5.08 (4.28-6.72)	5.52 (4.85-6.72)	4.77 (4.10-5.78)
sEng (22 -30 wks)	4.01(3.54-5.77)	3.85 (3.58-4.79)	4.18 (3.67-5.77)	3.79 (3.54-5.23)
sEng (32 -38 wks)	5.13(4.10-7.79)	5.33 (4.67-7.14)	5.33 (4.41-6.59)	5.32 (4.63-7.82)
sFlt1/PIGF (10-20 wks)	6.94 (3.36-13.46)	4.01 (1.36-8.93)	4.63(1.46-8.67)	6.94(2.27-13.86)
sFlt1/PIGF (22-30 wks)	2 (1.20-3.58)	2.21 (1.86-3.25)	2.00 (1.20-2.49)	2.47(1.58-5.67)
sFlt1/PIGF (32-38 wks)	1.99 (0.96-2.46)	1.73 (1.04-3.67)	1.81 (1.04-2.46)	1.93(0.90-3.82)
(sFlt1+sEng)/PIGF (10-20 wks)	33.97 (6.94-73.28)	22.22 (11.97-55.03)	19.09 (11.18-44.98)	29.05 (7.24-73.13)
(sFlt1+sEng)/PIGF (22-30 wks)	7.27 (4.34-8.15)	8.67 (6.00-12.83)	7.15 (5.03-12.24)	12.79 (4.34-23.16)
(sFlt1+sEng)/PIGF (32-38 wks)	5.99 (5.80-9.42)	8.16 (7.22-19.69)	7.55(6.56-10.93)	7.77(4.94-41.28)

*p<0.05 was considered statistically significant

Medians of the angiogenic profiles and selected clinical parameters, stratified by HIV status, are shown in Table 4. The PIGF levels in the HIV+ve group increased throughout the defined gestational intervals, in comparison to those who were HIV-ve. Whilst higher sFlt-1 levels was noted in the HIV+ve group during the 10-20 weeks gestational interval compared to the HIV-ve group, a reduction in their expression was observed during the latter gestational intervals. In contrast, sEng levels remained somewhat stable, but declined mid-gestation and thereafter stabilized to their initial expression levels (Table 4).

The indices of vascular disturbance (sFlt-1/PIGF and sFlt-1+sEng/PIGF), decreased considerably towards the end of the 2nd and 3rd gestational intervals in both the HIV+ve and -ve groups. However, the sFlt-1+sEng/PIGF ratio was much higher in the HIV+ve compared to the HIV-ve groups only in weeks 22-38. Additionally, the median hemoglobin levels was slightly higher in the HIV-ve group, whilst BMI and birth weight were almost similar (Table 4). Both the systolic and diastolic blood pressures were slightly higher in the HIV-ve groups throughout the defined gestational intervals compared to the HIV+ve groups.

Table 4: Angiogenic and antiangiogenic factors in normotensive pregnancy stratified by HIV status (n = 46)

Clinical characteristics	HIV -ve	HIV +ve
PlGF (pg/ml, median, range)		
10-20wks	240.4(162.8)	308.08 (346)
22-30wks	771.4 (374.27)	608.09 (428.8)
32-38wks	483.86 (488.25)	721.5 (595.98)
sFlt-1(pg/ml, median, range)		
10-20wks	819.69 (851.04)	1246.43 (799.57)
22-30wks	1335.78 (981.01)	1244.14 (935.09)
32-38wks	1663.97 (505.7)	1230.05 (857.52)
sEng (ng/ml, median, range)		
10-20wks	5.02 (5.64)	5.08 (1.75)
22-30wks	3.88 (1.97)	4.01 (1.69)
32-38wks	5.67 (2.37)	5.32 (3.38)
sFlt-1/PlGF (median, range)		
10-20wks	4 (3.58)	5.37 (11.3)
22-30wks	1.58 (2.81)	2.21 (1.39)
32-38wks	2.69 (2.55)	1.81 (1.5)
sFlt-1+sEng/PlGF (median, range)		
10-20wks	33.03 (54.54)	22.31 (38.5)
22-30wks	5.98 (12.5)	8.53 (6.92)
32-38wks	5.51 (3.09)	8.16 (5.4)
BMI (kg/m², mean, SD)	29.26 (3.16)	29.83 (7.32)
Hemoglobin (g/dL, mean, SD)	11.78 (0.87)	10.63 (1.54)
Birth weight (kg, mean, SD)	3.16(0.35)	2.98 (0.41)
Diastolic blood pressure (mmHg)		
10 -20 wks	63(9)	60 (2)
22 -30 wks	79 (16)	67(8)
32 - 38 wks	75 (16)	69(12)
Systolic blood pressure (mmHg)		
10 -20 wks	113 (30)	100 (10)
22 -30 wks	110(11)	106 (10)
32 - 38 wks	112 (24)	110 (10)

*p<0.05 was considered statistically significant

Correlations between angiogenic profiles and selected clinical characteristics at the defined gestational intervals are shown in Table 5. Maternal age was positively correlated with both indices of vascular disturbance (sFlt-1/PlGF and sFlt-1+sEng/PlGF) at 32-38 weeks' gestation ($p < 0.05$). In addition, a significant but negative correlation was noted between sFlt-1 and those with a BMI $> 25.00 \text{ kg/m}^2$ at 10-20 weeks. Despite the minimal increase noted in both systolic and diastolic blood pressure

throughout the defined gestational intervals, an inverse and significant correlation was observed between systolic blood pressure and PlGF and sFlt-1 at 32-38 weeks' gestation respectively. A statistically significant positive correlation ($r = 0.50$) was observed between diastolic blood pressure and the antiangiogenic ratio (sFlt-1/PlGF) at 32-38 weeks' gestation. A weak positive correlation was noted between hemaglobin and sFlt-1+sEng/PlGF ratio at 32-38 weeks' gestation.

Table 5: Pearson's correlations between clinical characteristics and angiogenic/antiangiogenic factors in normotensive pregnancies (n = 46)

Clinical factor	Angiogenic factor	Antiangiogenic factors		Antiangiogenic ratios (sFlt1 + sEng)/PIGF	
	PIGF	sFlt-1 (pg/ml)	sEng (ng/ml)	sFlt1/PIGF	sEng/PIGF
Maternal age (years)					
10-20wks	0,08	-0,29	0,1	-0,37	-0,31
22-32wks	-0,11	-0,09	0,02	-0,07	-0,06
32-38wks	-0,13	0,13	-0,1	0.61*	0.01*
BMI (kg/m²)					
<24.9					
10-20wks	0,09	-0,02	0,41	-0,43	-0,23
22-32wks	-0,36	-0,31	-0,22	0,19	-0,14
32-38wks	-0,03	0,19	0,41	0,85	0,6
>25.00					
10-20wks	0	-0.47*	-0,06	-0,19	0,04
22-32wks	0,08	-0,25	-0,27	-0,16	0,19
32-38wks	0,04	-0,2	-0,14	-0,33	0,06
Blood Pressure (mmHg)					
Systolic					
10-20wks	0,07	0,08	0,11	-0,1	-0,09
22-32wks	0,17	-0,19	-0,12	-0,19	-0,08
32-38wks	-0.38*	-0.47*	-0,11	0,29	0,34
Diastolic					
10-20wks	-0,09	0,1	0,03	0,01	-0,03
22-32wks	-0,04	-0,11	0,28	-0,02	0,23
32-38wks	-0,29	0,15	-0,24	0.50*	0,12
Hemoglobin/dL					
10-20wks	-0,31	0,17	0,14	0,23	0,1
22-32wks	-0,17	-0,17	0,06	0,11	-0,41
32-38wks	-0,01	0,04	-0,21	-0,16	0.02*

*p<0.05 was considered statistically significant

Discussion

This study demonstrates fluctuating angiogenic and antiangiogenic profiles (PIGF, sFlt-1, sEng, ratios sFlt-1:PIGF and sFlt-1+sEng/PIGF) at specific gestational intervals in a selected group of normotensive pregnant-women. The antiangiogenic ratio sFlt-1/PIGF ratio is suggestive of higher sFlt-1 and lower PIGF levels and is a recognized predictor of HDPs such as PE³⁶. Due to the growing association between circulating angiogenic and anti-angiogenic factors in HDPs, it is necessary to estimate their expressions throughout gestation and their link with risk factors even in normotensive pregnancies. To our knowledge, this is the first South African study to document the angiogenic concentrations at different stages of pregnancy. We previously reported higher se-

rum levels of the anti-angiogenic sFlt-1 and soluble endoglin in PE pregnancies compared with normotensive pregnancies irrespective of HIV status, however these were cross-sectional studies conducted at term prior to delivery^{33,34}.

Our current study investigated the combination of maternal risk factors and angiogenic profiles throughout the defined gestational intervals and intended to document the concentrations of angiogenic profiles in normotensive pregnancies, in a population that is prone to a high HIV antenatal prevalence. It is hoped that the concentrations documented in our study will assist as a reference range against which these angiogenic and anti-angiogenic factors may be compared in HIV complicated pregnancies.

Our data shows that changes in diastolic blood pressure, was positively correlated with the maternal anti-angiogenic ratio (sFlt-1/PlGF) at 32-38 weeks' gestation (Table 5). The mean systolic blood pressure measurements were inversely correlated with PlGF and sFlt-1 levels at 32-38 weeks' gestation. Similar angiogenic data was previously reported among normotensive pregnancies^{26,36}. Previous studies report an increased risk for PE development in healthy nulliparous women in response to elevated systolic and diastolic blood pressures^{28,37}. However, systolic and diastolic pressures measured at 13-21 weeks' gestation in pre-eclamptic women with high levels of sFlt-1 was reported to be similar to those in normal, uncomplicated pregnancies³⁶. This is suggestive of a healthy vasculature amongst a selected group of participants who were diagnosed with PE, indicative that PE development within this group occurred in response to either increased circulating sFlt-1 levels or increased susceptibility to vascular disorders. Thus, it is possible that even slight increases in blood pressure during early to mid gestation may result in vascular alterations. Variations in sFlt-1 during pregnancy in low risk women may be valuable for PE identification in those who lack the conventional risk factors.

Our results which demonstrate an increase PlGF and sFlt-1 around 32-38 weeks' gestation (Table 2) concur with other longitudinal studies^{19,28,38}. However, the increase appeared earlier in our study, at weeks 22-30 which concurred with Palm and co-workers³⁹, in contrast to other studies^{28,38}. The rising levels might be caused by a relative placental ischemia as a result of increasing myometrial tone and uterine contractions at the end of pregnancy in preparation for delivery⁴⁰. Moreover, it is possible that the modest expression of PlGF during the 10-20 weeks' gestational interval in our study may be attributed to the preliminary vasculogenic phases and thereafter progressively increases after 22 weeks' gestation due to the angiogenic switch from branching to nonbranching angiogenesis¹³. The rise in serum sFlt-1 levels observed in our study as gestation progresses, is consistent with several studies^{19,39,41}. It is likely that this elevation is due to increasing placental ischemia and oxidative stress occurring during pregnancy, indicative of angiogenic restriction, and controlled vascular permeability⁴², and is corroborated by others^{39,43,44}.

Our data also suggests a correlation of maternal age with the anti-angiogenic ratio at 32-38 weeks' gestation, which

is consistent with Staff and co-workers²⁷. However, our data shows no link between these angiogenic proteins with birth weight or hemoglobin levels, indicative that the circulating angiogenic alterations may have little or no effect on neonatal wellbeing, or that our sample size was too small. Nevertheless, our data has shown that hemoglobin levels were lower in the HIV+ve groups in comparison to the HIV-ve groups, despite the lack of statistical significance. In our study, the mean Hb level was 11.78 and 10.63 g/dL in HIV negative and positive women respectively. Anaemia in pregnancy is defined when the hemoglobin level in peripheral blood is < 11 g/dL⁴⁵, classified into mild anemia (10-10.9 g/dL), moderate anaemia (7-9.9 g/dL) and severe anaemia (< 7 g/dL)^{46,47}. Increased haemoglobin levels is shown to be correlated with elevated body mass index, primiparity, an increased risk of gestational hypertensive disorders and adverse birth outcomes such as low birth weight from third trimester onwards⁴⁸. In our study, we also identified lower hemoglobin levels in the HIV positive group, suggestive of mild anemia (Table 4). Recent provision of HAART to pregnant women, irrespective of their CD4 counts, has contributed significantly to the reduction in maternal deaths⁴. While HAART has the potential to lower the prevalence and severity of anaemia, mitochondrial toxicity and irregular reticulocyte counts appear to be common effects of zidovudine, which may exacerbate the risk of anemias⁴. A South African report published in 2014 indicate no difference in anemia prevalence in those on HAART in comparison to those who received zidovudine alone during pregnancy⁴⁹, but this warrants further investigation.

A reduction in serum levels of sFlt-1 and sEng in nulliparous women between 22-30 week's gestation, and an elevation from 32 weeks onwards was also noted in our study. Despite the lack of statistical significance, a reduction in serum sEng levels at 22-30 weeks' gestation and an elevation at 32 weeks' was observed (Table 2). Nulliparous pregnancies are characterized by higher ischemic placental environments since their spiral arteries have not been formerly remodeled and is therefore identified as a risk factor for PE development^{40,50}. It is still unclear why angiogenic/anti-angiogenic profiles are higher in nulliparous women. It is possible that the increased hypoxic environment increases placental sFlt-1 production⁴⁰. The high anti-angiogenic state observed in our study during

the 3rd gestational interval (32-38 weeks) is consistent with that reported by Bdolah and co workers⁵¹. The raised and modified angiogenic profile observed in nulliparous pregnant women may be a possible molecular tool that rationalizes the epidemiological association between PE and nulliparity. Sovio and co-workers also pooled the sFlt-1:PIGF ratio at 36 weeks' gestation, with maternal characteristics, and corroborated its clinical value in extrapolating the risk of PE development at term in unselected nulliparous women⁵².

BMI is also believed to be correlated with angiogenic balance even in uncomplicated pregnancies^{26,27}. In our study, the sFlt-1 levels decreased consistently throughout pregnancy amongst those with BMIs > 25 kg/m², however, a significant negative correlation was observed at 10-20 weeks. A similar relationship was observed by Mijal and co-workers between second trimester circulating sFlt-1 levels with BMI in normotensive pregnancies²⁰. Slight inconsistencies may prevail, since we pooled our 2nd trimester data into a gestational interval at 10-20 weeks, due to late antenatal attendance. Studies comparing BMI at the first antenatal consult with first and second trimester sFlt-1 levels and sFlt-1/PIGF ratio, revealed a positive correlation⁵³.

Conclusion

Since circulating concentrations of angiogenic and anti-angiogenic factors change with gestational age as shown in our study and various others^{19,26,27,54}, their inclusion at different gestation levels may expand its clinical value. However, due to the absence of pre-eclampsia cases in our study, our reference values for these angiogenic profiles are not intended for diagnostic use. The angiogenic concentrations described in our study may be used to explore the role of these angiogenic profiles in other placental-related disorders. Our data suggest that the angiogenic profiles during pregnancy may be differentially adjusted based on the varying physiological maternal demands. Our study was limited by a small sample size, which reduced statistical power to explore associations of clinical interest. These results should be confirmed in larger studies.

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Conflict of interest

The authors have no conflicts of interest to disclose.

Author contributions

All the authors contributed to the study, read and approved the final version, and consent to publication of this manuscript. MO: Laboratory analyses, data capture and management; PR: conception, design, statistical analyses and critical review of manuscript; MNS: conception and design of main study; LOC: conception and design of main study; DB: conception and design of main study; FH: conception and design of main study; SG: conception and design of main study; TN: conception and design of main study; NG: conception, design, analysis of data and writing of manuscript.

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