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Mid-pregnancy levels of angiogenic markers in relation to maternal characteristics

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Abstract

Objective—To describe relations among maternal demographic and lifestyle characteristics and mid-pregnancy levels of angiogenic markers (soluble Fms-like tyrosine kinase-1 (sFlt-1); placental growth factor (PlGF); soluble endoglin (sEng)).

Study Design—In a large pregnancy cohort, linear models were used to evaluate relations among maternal characteristics and mid-pregnancy angiogenic markers with and without covariate adjustment. Associations were examined in a sub-cohort including term and preterm deliveries (N = 1302) and among “normal” term pregnancies (N = 668).

Results—Concentrations of all factors declined with increasing maternal BMI. Multiparous women had lower sFlt-1 levels than primiparas. Higher PlGF and slightly lower sEng were observed among women who smoked at enrollment, but not among those who quit before enrollment. African-American women had higher levels of all markers.

Conclusions—Understanding relations among maternal characteristics and levels of angiogenic factors may improve studies utilizing these markers to examine etiologies and/or to predict adverse pregnancy outcome.

Keywords

preeclampsia; angiogenic factors; soluble endoglin; placental growth factor; soluble Fms-like tyrosine kinase-1

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DISCLOSURE: Discloser: Dr. Karumanchi is listed as a co-inventor on multiple patents assigned to the Beth Israel Deaconess Medical Center that are related to the diagnosis and therapy of preeclampsia. These patents have been licensed non-exclusively to multiple companies. Dr. Karumanchi is a consultant to Abbott, Beckman Coulter, Johnson & Johnson and Roche diagnostics.

Introduction

Altered patterns of angiogenic markers have been linked to pregnancy conditions responsible for significant maternal and fetal morbidity. These observations may offer etiological insights and translate into use of the markers for disease screening.^{1–4} For preeclampsia, the condition with the most extensive and strongest evidence, elevated concentrations of soluble Fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) and lower levels of placental growth factor (PlGF) have been detected months prior to disease onset.^{1,2,5,6} Additionally, several altered patterns of angiogenic markers may contribute to poor fetal growth,^{1,7} hypertension-related placental abruption,³ spontaneous preterm birth,^{7,8} proteinuria,⁹ gestational hypertension,⁶ and fetal death.^{7,10}

During pregnancy sFlt-1, PlGF and sEng are produced by placental tissue, although decidual cells, leukocytes, and other cells may contribute to overall levels.^{11–14} Measured in maternal serum, levels of sFlt1 and sEng are fairly steady early in pregnancy and rise more steeply later, whereas PlGF levels rise steadily into the third trimester and decline the last 8–10 weeks of pregnancy.¹ Angiogenic factors, either working independently or by modulating binding of VEGF and TGF- β to their receptors, may regulate placental angiogenesis.^{2,11,15} Additionally, these factors affect the maternal endothelium and, in cases of aberrant expression, lead to the development of symptoms like gestational hypertension and proteinuria.^{5,6,16,17} While linked to placental problems, it is unclear whether altered expression is cause or consequence of poor placental development and function.^{2,18,19}

Some studies have examined relations among these angiogenic markers and maternal characteristics,^{5,16,20–25} but many have been small, examined only one or two characteristics or markers, and lacked adjustment for other covariates. In addition, studies have varied in their inclusion/exclusion of complicated pregnancies. A more complete understanding of associations between angiogenic markers and maternal characteristics may provide clues to underlying mechanism, and may improve control of confounding in etiologic studies or those testing the screening/diagnostic potential of these markers.^{21,26,27}

In the Pregnancy Outcomes and Community Health Study (POUCH), we describe associations among maternal demographic, anthropometric, and lifestyle characteristics and each of three angiogenic markers measured in maternal serum at mid-pregnancy. Associations are examined within a group of “normal” term pregnancies in which women did not experience the most prevalent complications associated with altered angiogenic patterns, permitting a clearer interpretation of relations. For comparison we also examined associations among unselected pregnancies representing a spectrum of pregnancy outcomes, which may indicate the degree to which processes underlying poor pregnancy outcomes influence findings.

Materials and Methods

Population

The POUCH Study recruited pregnant women from 52 prenatal clinics in five different Michigan communities between September 1998 and June 2004. Eligible women had maternal serum α -fetoprotein (MSAFP) screening at 15–22 weeks gestation, had singleton pregnancies with no known abnormality or birth defect, were ≥ 15 years of age, were English-speaking, and had no diagnosis of pre-pregnancy diabetes mellitus. Given the study’s focus on preterm birth and previous reports linking elevated MSAFP to preterm delivery,^{28,29} all women with unexplained, elevated MSAFP levels (≥ 2.0 multiples of the median (MoM)) were invited to participate, enriching the sample for women with elevated

levels (7% vs. 3.5% in general obstetric population). A total of 3,038 women were enrolled between 15 and 27 weeks' gestation, at which time self-completed questionnaires and in-person interviews were administered and maternal biologic samples were collected. In total, 3019 women (99.5%) were followed through delivery and comprise the POUCH cohort. The POUCH Study protocol was approved by IRBs at Michigan State University, the Michigan Department of Community Health, and all delivery hospitals.

Study Sample

To utilize resources in a way that maximized statistical power for studying preterm delivery and at-risk groups like women with elevated MSAFP and African-Americans, we established a sub-cohort of women (N = 1371) in which more detailed information was obtained. The sub-cohort included all women who delivered preterm, all women with elevated MSAFP, and a sample of term births with normal MSAFP levels and oversampling of African American women. To account for oversampling of preterm deliveries and at-risk groups, analyses were weighted according to the probabilities of selection into the cohort and sub-cohort. (E.g.-the proportion of individuals with high MSAFP in the entire cohort was approximately 7%, twice that of the general population, so this group would be assigned a cohort adjustment weight of 1/2. Additional weights would be calculated for sampling into the subcohort.) The weighted results should reflect the experience of the population sampled, as previously demonstrated for demographic characteristics and selected pregnancy outcomes.³⁰

Women included in this analysis belonged to the sub-cohort, had measures of angiogenic factors available and were not missing information on important covariates (N = 1302) (See Figure 1). Additionally, we conducted a subset analysis with women who had "normal" pregnancies defined as: 1) term delivery (>37 weeks gestational age); 2) MSAFP < 2.0 MoM; 3) delivery of a child who was not small-for-gestational age (SGA), defined < 10th percentile of weight for gestational age;³¹ 4) no diagnosis of any hypertensive disorder. Hypertensive disorders were ascertained through review of prenatal and labor/delivery records by trained abstractors and included diagnoses of chronic hypertension prior to pregnancy, gestational hypertension (minimal criteria: DBP ≥ 90 or SBP ≥ 140 on two different days after 20 weeks gestation without evidence of proteinuria) or preeclampsia (minimal criteria: gestational hypertension plus proteinuria defined as 2+ protein on urine dipstick once or 1+ protein on two occasions after 20 weeks gestation. If proteinuria was present before 20 weeks, increased levels after 20 weeks were considered indicative of preeclampsia).

Covariates

Maternal demographic, reproductive, lifestyle, and anthropometric characteristics were obtained via interview or questionnaire administered at enrollment. Race was categorized for these analyses as African American or non-African American. Medicaid enrollment (yes/no) and completed years of education were proxies for socio-economic status. Smoking was grouped into four-levels: did not smoke during pregnancy, smoked in pregnancy but stopped prior to enrollment, smoked < half pack per day at enrollment, and smoked ≥ half pack per day at enrollment. Pre-pregnancy weight and maternal height were obtained from the questionnaire. These two measures were used to calculate pre-pregnancy body mass index (BMI) which was grouped according to CDC guidelines into underweight, normal, overweight and obese.³² Gestational age (GA) at study enrollment, grouped as 15–<20, 20–<25, and 25–<28 weeks, was based on LMP if this estimate was within 2 weeks of the date estimated from a <25 week ultrasound. Otherwise ultrasound dates were utilized.

Measurement of angiogenic markers

Maternal serum samples collected at enrollment were stored at -80°C . For analysis samples were randomly allocated to batches by study site and length of post-collection storage time. Additionally, all batches contained the same proportion of preterm deliveries since this outcome was a primary focus of the study. Measurements of sFlt-1, PlGF, and sEng were made using commercially available ELISA kits (R&D Biosystems, Minneapolis, MN) by one Karumanchi Laboratory member blinded to patients' clinical data. All measurements were performed in duplicate. Assays were re-run if measures differed by more than 25%. Limits of detection for each marker were: 7 pg/mL (sFlt-1), 5 ng/mL (PlGF), and 5 pg/mL (sEng). Inter-assay coefficients of variation were 7.6%, 10.9%, and 3.0% for sFlt-1, PlGF and sEng respectively. Intra-assay coefficients were 3.3%, 5.6%, and 6.3% for the same. Duplicate measures were averaged and log-transformed to create approximately normal distributions for use in general linear models.

Analysis

Associations between angiogenic markers and maternal characteristics were examined in two groups of women: all POUCH pregnancies with complete biomarker and covariate measurements (95% of sub-cohort women, $N = 1302$) and a sample of "normal" pregnancies (term, normal MSAFP, no hypertensive disorders, not SGA, $N = 668$). Analyses were completed in SAS 9.1.3 or 9.2 and were appropriately weighted using Survey Procedures. Frequency distributions were calculated using Proc SurveyFreq. Linear regression was implemented in Proc SurveyReg to determine the regression coefficient for each angiogenic marker regressed on covariates of interest. Covariates were examined individually and in multivariable models. Unadjusted and adjusted least squares means and 95% confidence intervals are presented for each factor at different levels of maternal demographic, lifestyle and anthropometric variables.

Results

The weighted frequencies showed little difference between the distributions of maternal characteristics using the entire POUCH sub-cohort and women with complete measurements (Table 1). Approximately 24% of women were African American, slightly more than half were 20–29 years of age, 41% were primiparas, > 25% reported smoking at some time during pregnancy, and most enrolled between 20 and 24 weeks' gestation at a mean 22.4 and 22.5 gestational weeks for the total and "normal" pregnancy samples respectively (Table 1).

Associations among maternal characteristics and angiogenic marker levels are presented without adjustment (Tables 2 and 3: total sample and "normal" pregnancies respectively) and with adjustment (Tables 4 and 5: total sample and "normal" pregnancies respectively). The text focuses on findings from the "normal" pregnancy sample.

Gestational Age

Gestational age was positively associated with PlGF in all models, a trend toward a positive association was observed for sEng, and sFlt-1 levels were not significantly different across the 15–27 week gestational age window.

Parity and Maternal Age

In unadjusted models, multiparity was significantly associated with lower sEng and sFlt-1 levels. After adjustment, multiparous women still had lower levels than primiparas, but only sFlt-1 results retained statistical significance (1756 pg/mL (95% CI: 1581–1949); 2148 pg/mL (95% CI: 1899–2429), $p < 0.01$, Table 5). Maternal age, a factor correlated with parity,

was not associated with biomarker concentration after adjustment. This was true for models utilizing ≥ 35 as the cut-off for “older” mothers.

Socioeconomic Status

Medicaid use was associated with lower sEng and higher PIGF levels only in the unadjusted total sample (Table 2). No strong associations were observed for Medicaid use among “normal” pregnancies (Table 5). Similarly, educational attainment and PIGF levels were not associated after adjustment.

BMI

Consistently, pre-pregnancy BMI was inversely associated with levels of each marker. sEng levels differed significantly between normal (5.63 pg/mL) and obese women (4.90 pg/mL, $p < 0.01$, Table 5). PIGF demonstrated marked changes and a monotonic increase across BMI categories: (underweight = 606.2 ng/mL, normal = 523.0 ng/mL, overweight = 457.9 ng/mL, and obese = 345.6 ng/mL (Table 5); adjusted β for the continuous BMI variable = $-0.027/\text{BMI unit}$, $p < 0.0001$). A slightly weaker inverse association was observed between pre-pregnancy weight and angiogenic levels.

Smoking

Among women who smoked at enrollment, unadjusted sEng levels were lower and PIGF concentrations were higher than those of non-smokers, with some dose-response suggested. After adjustment, only the PIGF association persisted, levels in “normal” pregnancies being 404.3 ng/mL, 564.9 ng/mL, and 610.1 ng/mL for non-smokers, those who smoked $< 1/2$ pack/day, and smokers of $\geq 1/2$ pack/day, respectively ($P < 0.01$ comparisons vs. non-smokers (Table 5)). For each marker, levels in women who smoked during pregnancy but quit before enrollment were indistinguishable from levels in non-smokers.

Race

For each factor, African Americans had higher levels of angiogenic markers than women of all other racial/ethnic groups and covariate adjustment only strengthened associations (Tables 2–5). Among “normal” pregnancies PIGF levels were 34% higher in African Americans ($p < 0.01$) and sFlt-1 concentrations were 27% greater ($p < 0.01$ (Table 5)). Findings were similar for analyses limited to African-American and non-Hispanic White women.

Comment

We examined associations among established risk factors for adverse pregnancy outcomes^{2,33} and levels of angiogenic markers in women from the POUCH Study. Our findings were consistent with overall patterns previously reported for gestational age, parity, maternal age and BMI. In addition, we found levels of all three markers varied by race and concentrations were similar in women who quit smoking during pregnancy and those who did not smoke in pregnancy.

Studies that have measured angiogenic markers at different gestational weeks have noted a rise in PIGF levels at mid-pregnancy,^{1,5,16} little change in sFlt-1 across mid-pregnancy but elevations later in pregnancy,^{1,5,7,22,34} and increasing sEng levels with increasing GA.^{1,6} Overall, the patterns observed in our sample were similar to those from previous studies covering the period of mid-pregnancy.

Maternal age and parity have been examined explicitly in a few, small studies and have yielded inconsistent results. Associations between maternal age and first trimester levels of

sFlt-1 and PlGF have been inconsistent,^{16,35} whereas for levels at delivery only PlGF was associated with maternal age.²⁰ In contrast, univariable analyses demonstrated higher sFlt-1 and lower PlGF for nulliparas irrespective of gestational weeks at measurement.^{16,20,35} After adjustment, maternal age was not associated with first trimester sFlt-1 or PlGF,^{21,35} however, parity was associated with both lower sFlt-1 and higher PlGF in a small sample³⁵ and with higher PlGF in a larger one.²¹

In POUCH, younger women and primiparas tended to have higher sFlt-1 and sEng levels, but after adjustment only primiparity was associated with higher sFlt-1. These results are consistent with others except that we did not observe a positive association between multiparity and PlGF. This may reflect differences in the timing of PlGF measurement, sample size, or covariates used for adjustment. Our covariate adjustment was more extensive than one small, positive study³⁵ and did not include measures of infant growth like the second.²¹ While the associations with parity need confirmation, other data support these findings. First, high sFlt-1 and sEng characterize preeclampsia, a disease more likely to complicate first pregnancies.^{5,6,23} Second, a small longitudinal study reported significant declines in first trimester sFlt-1 between first and second pregnancies.²⁴

We are unaware of other studies examining relations between socioeconomic status (SES) and angiogenic markers. In POUCH, some angiogenic marker levels were associated with educational attainment and history of Medicaid enrollment, two proxies for SES, but only in unadjusted models. Overall, our results suggest that the SES-angiogenic marker association reflects correlations between SES variables and other maternal characteristics.

Overall, studies examining the association between maternal BMI/weight and angiogenic markers find statistically significant inverse relations, although findings have not been consistent across studies or markers.^{5,7,16,20,21,35} We observed consistent inverse associations with all three biomarkers, even among “normal” pregnancies which suggests this pattern is not related to a high-risk subset. A dilution effect may explain the inverse relation with maternal BMI/weight; similar effects are thought to occur with substances crossing from the fetus (e.g. MSAFP)^{36,37} or placenta (e.g. corticotrophin releasing hormone)^{37,38} into the maternal circulation. Given that high BMI/obesity is a risk factor for preeclampsia,^{2,33} the relation between angiogenic markers, BMI and pregnancy outcome requires further study. Among women with high BMI and preeclampsia, levels of angiogenic markers in pregnancy might reflect a combination of effects due to larger maternal size, decreased pregnancy-related vascular expansion and endothelial dysfunction.^{33,39–42}

Given that smoking protects against preeclampsia and angiogenic markers are differentially expressed in preeclampsia (higher sFlt-1, lower PlGF, higher sEng),^{2,5,6,43} it follows that women who smoke during pregnancy appear to have a more favorable angiogenic profile (lower sFlt-1, higher PlGF, lower sEng) than women who do not. Two studies, one presenting unadjusted estimates and the other levels in matched cases and controls, found third trimester sFlt-1 levels were lower among women who smoked during pregnancy vs. those who did not.^{6,22,44} In a third study examining levels between 33–42 weeks gestation, findings were no longer statistically significant after covariate adjustment.⁴⁵ For measures taken earlier in pregnancy, results were less consistent.^{7,22,45} Measured anytime during pregnancy, PlGF was higher among women who smoked in pregnancy.^{6,7,21,46} Unadjusted and adjusted sEng was lower among current smokers before 20 weeks gestation, but not later in pregnancy.^{6,45}

Consistent with previous reports, we found statistically-significantly higher PlGF levels and non-significantly lower sEng levels among actively smoking women. We may have been

unable to detect differences in sFlt-1 levels due to the timing of our measurement in early-mid second trimester (15% at <20 weeks, most 20–24.9 weeks). The smoking effect may be greater later in pregnancy due to dramatic changes in angiogenic marker levels at the end of pregnancy.⁴⁷ Even for preeclampsia, changes in sFlt-1 levels are inconsistently detected < 20 weeks GA.^{6,34} While covariate adjustments varied between previous studies, similar findings in our unadjusted and adjusted models make differences in covariate adjustment an unlikely explanation for our result.

Unlike others, we examined levels of angiogenic markers separately for women who smoked early in pregnancy, but stopped before enrollment. Angiogenic marker levels among these smokers who quit during pregnancy are no different from those of women who did not smoke during pregnancy. While we lack biochemical confirmation of smoking status, misclassification may be greater among women who report quitting during pregnancy,⁴⁸ with active smokers misreporting themselves as having quit. This type of misclassification would, in expectation, reduce sFlt-1 and sEng levels and elevate PIGF levels; correction would likely strengthen our findings. Larger studies are needed to confirm these observations. However, our findings are consistent with those of a report examining preeclampsia risk by pattern of smoking. Smokers who quit between their first antenatal visit (<15 weeks) and 30–32 weeks gestation were as likely to develop preeclampsia as women who did not smoke in pregnancy, whereas women who reported smoking at 30–32 weeks, independent of prior smoking in pregnancy, were less likely to have preeclampsia.⁴⁹

Race is among the least studied characteristics with respect to angiogenic markers. Studies from England reported adjusted first trimester levels of PIGF were higher among black women as compared to white women.^{21,26} We are unaware of other large studies that have explicitly reported differences in mean biomarker levels among women of different racial/ethnic groups. Our findings confirm and extend the previous PIGF findings, showing sFlt-1 and sEng levels were also higher in African-American women vs. non-Hispanic Whites. While different populations were studied and modeling approaches varied, the PIGF β -coefficient for race in our model (15–27 weeks GA), was nearly twice that reported previously (evaluated at 11–14 weeks GA). Differences in angiogenic marker levels by race/ethnicity should be explored more fully, describing trajectories for each factor through pregnancy and potential interactions with other maternal characteristics such as parity which may modify race-preeclampsia associations.⁵⁰

The strengths of this study include its size, the clearly defined population studied, its use of a “normal” pregnancy group and a comparison total pregnancy sample, adjustment for multiple covariates, and its exploration of little-studied characteristics. A significant limitation is having only one biomarker measurement during pregnancy. The importance of longitudinal changes in angiogenic factors has been clearly shown^{1,8,18,51,52} and we may not have taken measures at the optimal time for detecting all associations with biomarker levels. Finally, examining relations among multiple angiogenic markers and many maternal characteristics increases the probability of a false positive finding. We did not adjust for multiple comparisons since the characteristics examined were chosen *a priori*, mostly based on previously reported associations and results from other pregnancy-related biomarkers.

This analysis confirms several previously reported associations between angiogenic markers and maternal characteristics, including lower sFlt-1 levels among multiparas and the inverse relation between all markers and maternal BMI. Additionally, we demonstrate that levels of all angiogenic markers are higher among African-American women. Examining profiles in women according to smoking during pregnancy, we report higher PIGF levels and a trend toward lower sEng among current smokers, but not for smokers who quit earlier in pregnancy. These findings can inform the selection of covariates included in models

utilizing angiogenic biomarkers and suggest new avenues for research examining the contribution of angiogenic factors to the etiology of adverse pregnancy outcomes.

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Figure 1.
Flow diagram showing the derivation of the Total Pregnancy Sample and the “Normal” Pregnancy Sample from the entire POUCH cohort

Table 1
Characteristics of the entire sub-cohort, total pregnancy sample and normal pregnancy sample

Characteristics	Entire Sub-cohort (N = 1371)		Total Pregnancies Sample (N= 1302)		“Normal” Pregnancy Sample (N= 668)	
	N	Weighted %	N	Weighted %	N	Weighted %
Maternal Characteristics						
Race						
White	692	65.8	668	65.7	316	66.8
African American	579	24.6	536	24.6	305	23.3
Asian	23	2.2	22	2.1	9	1.9
Hispanic	58	5.1	58	5.3	26	5.5
Other	19	2.3	18	2.4	12	2.5
Age						
<20 years	243	14.7	227	14.6	113	13.2
20–29 years	776	57.2	734	56.8	401	59.0
≥30 years	352	28.1	341	28.6	154	27.8
Parity						
Primiparous	577	41.5	543	41.0	256	38.3
Multiparous	793	58.5	759	59.0	412	61.7
Missing	1	0.0	-	-	-	-
Education (highest level attained)						
<12 th grade	317	18.9	296	18.8	154	17.7
12 th grade	388	27.0	367	27.1	184	26.1
>12 th grade	666	54.1	639	54.1	330	56.2
Medicaid						
No	586	50.9	565	50.8	286	52.6
Yes	783	49.1	737	49.2	382	47.4
Missing	2	0.01	-	-	-	-
Pre-Pregnancy Body Mass Index						
Underweight (< 18.5)	65	3.9	59	3.8	25	3.3
Normal (18.5–24.9)	609	46.6	581	46.6	295	47.4

Characteristics	Entire Sub-cohort (N = 1371)		Total Pregnancies Sample (N= 1302)		“Normal” Pregnancy Sample (N= 668)	
	N	Weighted %	N	Weighted %	N	Weighted %
Characteristics						
Overweight (25.0–29.9)	303	23.0	290	23.2	163	24.3
Obese (≥30)	394	26.4	372	26.4	185	25.1
Smoking						
Never during pregnancy	979	72.5	933	72.5	494	75.6
Quit before enrollment	132	9.9	125	9.9	55	8.7
Current, < ½ pack per day	182	11.5	167	11.4	84	9.8
Current, ≥ ½ pack per day	78	6.1	77	6.3	35	5.9
Pregnancy Characteristics						
Gestational Age at Enrollment						
15–19.9 weeks	225	15.8	211	15.6	102	14.1
20–24.9 weeks	964	71.1	915	71.2	482	73.2
25–27.9 weeks	182	13.2	176	13.3	84	12.6
Fetal Growth						
Small for Gestational Age	150	9.4	138	9.3	-	-
Not Small for Gestational Age	1219	90.5	1162	90.6	668	100
Missing	2	0.1	2	0.1	-	-
Hypertensive Disorders						
Gestational Hypertension/Preeclampsia ^a	100	6.6	100	6.9	-	-
Chronic Hypertension	49	3.0	45	3.0	-	-
Normotensive	1222	90.3	1157	90.0	668	100.0

^aPreeclampsia includes all cases including those superimposed on chronic hypertension.

Unadjusted least squares mean concentrations of angiogenic markers by maternal and pregnancy characteristics – Total pregnancy sample (N = 1302)

Table 2

	Placental Growth Factor (PIGF)			Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)		
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI
Gestational Age at Enrollment									
15– 19.9 weeks (ref)	211	228.2	(207.9, 250.4)	ref	5.30	(5.06, 5.55)	ref	1670	(1504, 1855)
20– 24.9 weeks	915	381.5	(363.6, 400.3)	< 0.01	5.28	(5.17, 5.40)		1669	(1593, 1748)
25– 27.9 weeks	176	556.8	(509.4, 608.6)	< 0.01	5.81	(5.51, 6.13)	< 0.01	1676	(1501, 1871)
Parity									
Primiparous (ref)	543	378.5	(354.6, 403.9)	ref	5.61	(5.45, 5.78)	ref	1948	(1839, 2063)
Multiparous	759	364.7	(344.9, 385.7)		5.18	(5.06, 5.30)	< 0.01	1501	(1425, 1581)
Maternal Age at Enrollment									
<20 years (ref)	227	428.9	(386.4, 476.0)	ref	5.59	(5.28, 5.91)	ref	2002	(1820, 2201)
20– 29 years	734	358.1	(339.3, 378.0)	< 0.01	5.29	(5.17, 5.41)		1586	(1503, 1673)
≥ 30 years	341	367.0	(336.4, 400.5)	< 0.05	5.36	(5.16, 5.56)		1688	(1571, 1814)
Medicaid									
No (ref)	565	354.0	(333.1, 376.2)	ref	5.46	(5.32, 5.61)	ref	1702	(1609, 1802)
Yes	737	387.9	(365.6, 411.6)	< 0.05	5.24	(5.11, 5.38)	< 0.05	1637	(1550, 1730)
Education									
Grade < 12	296	449.5	(414.6, 487.3)	< 0.01	5.32	(5.11, 5.54)		1704	(1550, 1874)
Grade = 12 (ref)	367	347.1	(317.8, 379.1)	ref	5.39	(5.20, 5.60)	ref	1631	(1513, 1757)
Grade > 12	639	357.5	(337.5, 378.8)		5.34	(5.21, 5.48)		1678	(1590, 1771)
Pre-pregnancy Body Mass Index									
Underweight	59	555.4	(470.8, 655.2)	< 0.01	5.33	(4.78, 5.94)		1939	(1604, 2345)
Normal (ref)	581	412.6	(387.3, 439.7)	ref	5.57	(5.42, 5.72)	ref	1884	(1782, 1993)
Overweight	290	378.0	(347.3, 411.3)		5.43	(5.25, 5.62)		1633	(1513, 1764)
Obese	372	283.4	(263.8, 304.4)	< 0.01	4.93	(4.75, 5.12)	< 0.01	1347	(1245, 1458)
Smoking									
Didn't smoke during pregnancy (ref)	933	350.2	(333.7, 367.5)	ref	5.41	(5.30, 5.53)	ref	1700	(1623, 1781)
Stopped smoking before enrollment	125	317.6	(275.2, 366.5)		5.47	(5.14, 5.83)		1618	(1420, 1844)

	Placental Growth Factor (PlGF)				Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)		
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b
< Half pack/ day	167	507.3	(451.8, 569.7)	< 0.01	5.17	(4.98, 5.38)	< 0.05	1628	(1451, 1826)	
≥ Half pack/ day	77	509.4	(437.6, 593.0)	< 0.01	4.83	(4.40, 5.30)	< 0.05	1493	(1277, 1745)	
Race										
Whites/ others (ref)	766	348.9	(330.9, 367.9)	ref	5.31	(5.18, 5.43)	ref	1591	(1514, 1673)	ref
African Americans	536	444.2	(419.6, 470.2)	< 0.01	5.50	(5.36, 5.64)	< 0.05	1936	(1839, 2038)	< 0.01

CI = Confidence Interval

^aLeast square means of the log biomarker value, back transformed to normal scale

^bRef denotes referent category for comparisons

Unadjusted least squares mean concentrations of angiogenic markers by maternal and pregnancy characteristics – Normal pregnancy sample (N = 668)

Table 3

	Placental Growth Factor (PIGF)			Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)			
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b
Gestational Age at Enrollment										
15– 19.9 weeks (ref)	102	239.5	(212.0, 270.5)	ref	5.23	(4.93, 5.54)	ref	1663	(1440, 1920)	ref
20– 24.9 weeks	482	388.0	(366.2, 411.1)	<0.01	5.25	(5.12, 5.38)		1691	(1602, 1786)	
25– 27.9 weeks	84	565.2	(505.4, 632.0)	<0.01	5.55	(5.23, 5.88)		1605	(1409, 1827)	
Parity										
Primiparous (ref)	256	399.8	(368.1, 434.3)	ref	5.48	(5.30, 5.68)	ref	1969	(1840, 2106)	ref
Multiparous	412	368.3	(344.8, 393.3)		5.16	(5.02, 5.31)	<0.01	1517	(1425, 1614)	<0.01
Maternal Age at Enrollment										
<20 years (ref)	113	465.4	(407.2, 532.0)	ref	5.41	(5.08, 5.75)	ref	2155	(1945, 2388)	ref
20– 29 years	401	362.9	(340.1, 387.2)	<0.01	5.27	(5.13, 5.41)		1584	(1486, 1689)	<0.01
≥ 30 years	154	380.7	(342.9, 422.7)	<0.05	5.26	(5.02, 5.51)		1677	(1539, 1827)	<0.01
Medicaid										
No (ref)	286	365.9	(340.7, 393.0)	ref	5.39	(5.23, 5.55)	ref	1672	(1564, 1788)	ref
Yes	382	396.3	(367.8, 427.1)		5.17	(5.01, 5.33)		1680	(1571, 1797)	
Education										
Grade < 12	154	461.3	(416.2, 511.4)	<0.01	5.22	(4.98, 5.46)		1869	(1678, 2082)	
Grade = 12 (ref)	184	348.4	(312.2, 388.8)	ref	5.35	(5.12, 5.59)	ref	1623	(1474, 1788)	ref
Grade > 12	330	372.2	(347.7, 398.5)		5.27	(5.12, 5.43)		1644	(1543, 1751)	
Pre-pregnancy Body Mass Index										
Underweight	25	560.3	(442.9, 708.9)	<0.05	5.63	(4.94, 6.43)		1985	(1560, 2525)	
Normal (ref)	295	427.3	(396.1, 460.8)	ref	5.53	(5.36, 5.71)	ref	1912	(1789, 2043)	ref
Overweight	163	380.8	(345.9, 419.2)		5.30	(5.09, 5.51)		1582	(1443, 1734)	<0.01
Obese	185	289.0	(263.5, 317.0)	<0.01	4.79	(4.60, 4.99)	<0.01	1352	(1232, 1485)	<0.01
Smoking										
Didn't smoke during pregnancy (ref)	494	362.4	(341.8, 384.1)	ref	5.34	(5.21, 5.48)	ref	1661	(1571, 1756)	ref
Stopped smoking before enrollment	55	334.1	(274.7, 406.5)		5.31	(4.91, 5.75)		1583	(1337, 1875)	

	Placental Growth Factor (PlGF)				Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)		
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b
< Half pack/ day	84	527.0	(452.3, 613.9)	< 0.01	5.07	(4.85, 5.30)	< 0.05	1866	(1652, 2109)	
≥ Half pack/ day	35	491.3	(420.8, 573.7)	< 0.01	4.91	(4.45, 5.41)		1714	(1435, 2049)	
Race										
Whites/ others (ref)	363	356.8	(334.9, 380.1)	ref	5.25	(5.12, 5.40)	ref	1599	(1508, 1694)	ref
African Americans	305	467.5	(435.1, 502.4)	< 0.01	5.38	(5.22, 5.55)		1958	(1830, 2095)	< 0.01

CI = Confidence Interval

^aLeast square means of the log biomarker value, back transformed to normal scale

^bRef denotes referent category for comparisons

Adjusted least squares mean concentrations of angiogenic markers by maternal and pregnancy characteristics – Total pregnancy sample (N= 1302)

Table 4

	Placental Growth Factor (PlGF)			Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)		
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI
Gestational Age at Enrollment									
15– 19.9 weeks (ref)	211	288.5	(261.5, 318.1)	ref	5.41	(5.09, 5.75)	ref	1902	(1698, 2132)
20– 24.9 weeks	915	491.6	(457.3, 528.4)	< 0.01	5.31	(5.09, 5.53)		1829	(1695, 1973)
25– 27.9 weeks	176	710.9	(641.5, 787.9)	< 0.01	5.79	(5.44, 6.17)		1816	(1599, 2062)
Parity									
Primiparous (ref)	543	463.5	(427.8, 502.2)	ref	5.68	(5.43, 5.95)	ref	2060	(1872, 2267)
Multiparous	759	467.3	(430.5, 507.2)		5.32	(5.05, 5.60)	< 0.01	1659	(1521, 1810)
Maternal Age at Enrollment									
<20 years (ref)	227	465.0	(412.2, 524.6)	ref	5.53	(5.14, 5.96)	ref	1939	(1716, 2191)
20– 29 years	734	450.0	(417.7, 484.8)		5.45	(5.20, 5.70)		1737	(1601, 1885)
≥ 30 years	341	481.8	(434.8, 533.8)		5.52	(5.22, 5.84)		1876	(1675, 2102)
Medicaid									
No (ref)	565	459.3	(419.7, 502.7)	ref	5.61	(5.30, 5.93)	ref	1887	(1711, 2080)
Yes	737	471.6	(437.2, 508.8)		5.40	(5.16, 5.65)		1812	(1653, 1985)
Education									
Grade < 12	296	484.4	(440.0, 533.3)		5.47	(5.18, 5.78)		1757	(1577, 1958)
Grade = 12 (ref)	367	444.5	(404.7, 488.2)	ref	5.62	(5.32, 5.94)	ref	1916	(1732, 2120)
Grade > 12	639	468.2	(427.8, 512.4)		5.41	(5.11, 5.73)		1876	(1687, 2087)
Pre-pregnancy Body Mass Index									
Underweight	59	609.3	(516.6, 718.5)	< 0.05	5.46	(4.88, 6.11)		2032	(1672, 2470)
Normal (ref)	581	499.9	(464.8, 537.7)	ref	5.74	(5.49, 5.99)	ref	2064	(1900, 2242)
Overweight	290	454.1	(412.8, 499.5)	< 0.05	5.67	(5.41, 5.94)		1854	(1689, 2035)
Obese	372	339.3	(312.4, 368.5)	< 0.01	5.16	(4.91, 5.41)	< 0.01	1502	(1363, 1655)
Smoking									
Didn't smoke during pregnancy (ref)	933	387.2	(363.4, 412.6)	ref	5.61	(5.42, 5.81)	ref	1861	(1735, 1996)
Stopped smoking before enrollment	125	352.7	(312.0, 398.8)		5.70	(5.31, 6.11)		1808	(1578, 2071)

	Placental Growth Factor (PIGF)				Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)		
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b
< Half pack/ day	167	551.5	(493.1, 616.7)	< 0.01	5.42	(5.16, 5.69)		1835	(1625, 2071)	
≥ Half pack/ day	77	623.0	(533.6, 727.4)	< 0.01	5.29	(4.78, 5.84)		1893	(1586, 2259)	
Race										
Whites/ others (ref)	766	405.8	(376.7, 437.0)	ref	5.33	(5.08, 5.59)	ref	1627	(1489, 1778)	ref
African Americans	536	533.9	(489.3, 582.5)	< 0.01	5.67	(5.39, 5.97)	< 0.01	2100	(1911, 2307)	< 0.01

CI= Confidence Interval

^aLeast square means of the adjusted log biomarker value, back transformed to normal scale. Least squares means are adjusted for all other covariates listed in the table

^bRef denotes referent category for comparisons

Adjusted least square mean concentrations of angiogenic markers by maternal and pregnancy characteristics – Normal pregnancy sample (N = 668)

Table 5

	Placental Growth Factor (PIGF)			Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)			
	N	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b	Mean ^a	95% CI	p ^b
Gestational Age at Enrollment										
15– 19.9 weeks (ref)	102	301.7	(263.1, 346.0)	ref	5.35	(4.95, 5.78)	ref	2001	(1729, 2317)	ref
20– 24.9 weeks	482	495.6	(451.3, 544.3)	< 0.01	5.29	(5.04, 5.56)		1967	(1794, 2157)	
25– 27.9 weeks	84	709.0	(623.7, 805.9)	< 0.01	5.54	(5.16, 5.94)		1859	(1583, 2183)	
Parity										
Primiparous (ref)	256	474.0	(425.8, 527.7)	ref	5.51	(5.21, 5.82)	ref	2148	(1899, 2429)	ref
Multiparous	412	472.6	(424.5, 526.0)		5.28	(4.98, 5.60)		1756	(1581, 1949)	< 0.01
Maternal Age at Enrollment										
<20 years (ref)	113	493.3	(419.5, 580.0)	ref	5.42	(5.00, 5.88)	ref	2109	(1820, 2443)	ref
20– 29 years	401	448.6	(406.8, 494.7)		5.40	(5.10, 5.72)		1787	(1608, 1985)	< 0.05
≥ 30 years	154	479.1	(418.5, 548.4)		5.36	(5.01, 5.73)		1943	(1685, 2240)	
Medicaid										
No (ref)	286	462.5	(412.9, 518.1)	ref	5.49	(5.15, 5.85)	ref	1965	(1732, 2230)	ref
Yes	382	484.3	(436.3, 537.6)		5.30	(5.03, 5.59)		1918	(1717, 2144)	
Education										
Grade < 12	154	486.4	(427.8, 553.0)		5.32	(4.97, 5.69)		1896	(1664, 2161)	
Grade = 12 (ref)	184	452.2	(399.1, 512.5)	ref	5.57	(5.22, 5.95)	ref	2005	(1755, 2291)	ref
Grade > 12	330	482.0	(429.0, 541.5)		5.30	(4.96, 5.66)		1925	(1690, 2193)	
Pre-pregnancy Body Mass Index										
Underweight	25	606.2	(478.2, 768.4)		5.65	(4.91, 6.49)		2115	(1669, 2681)	
Normal (ref)	295	523.0	(475.6, 575.1)	ref	5.63	(5.35, 5.94)	ref	2232	(2012, 2477)	ref
Overweight	163	457.9	(407.3, 514.9)	< 0.05	5.43	(5.14, 5.74)		1905	(1693, 2145)	< 0.01
Obese	185	345.6	(311.0, 384.2)	< 0.01	4.90	(4.66, 5.16)	< 0.01	1580	(1396, 1788)	< 0.01
Smoking										
Didn't smoke during pregnancy (ref)	494	404.3	(371.2, 440.5)	ref	5.51	(5.27, 5.75)	ref	1829	(1676, 1996)	ref
Stopped smoking before enrollment	55	360.1	(301.0, 430.8)		5.49	(5.03, 6.00)		1785	(1503, 2120)	

	N	Placental Growth Factor (PIGF)			Soluble Endoglin (sEng)			Soluble Fms-Like Tyrosine Kinase-1 (sFlt-1)		
		Mean ^a	95% CI	P ^b	Mean ^a	95% CI	P ^b	Mean ^a	95% CI	P ^b
< Half pack/ day	84	564.9	(487.6, 654.3)	< 0.01	5.24	(4.95, 5.55)		2002	(1721, 2329)	
≥ Half pack/ day	35	610.1	(510.2, 729.6)	< 0.01	5.34	(4.79, 5.95)		2175	(1777, 2661)	
Race										
Whites/ others (ref)	363	409.2	(369.5, 453.2)	ref	5.25	(4.97, 5.55)	ref	1723	(1544, 1921)	ref
African Americans	305	547.4	(489.9, 611.7)	< 0.01	5.54	(5.22, 5.87)	< 0.05	2189	(1943, 2466)	< 0.01

CI= Confidence Interval

^aLeast square means of the adjusted log biomarker value, back transformed to normal scale. Least squares means are adjusted for all other covariates listed in the table

^bRef denotes referent category for comparisons