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Maternal Angiogenic Profile in Pregnancies that Remain Normotensive

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Abstract

Objective—We sought to determine if maternal characteristics are associated with angiogenic profile in the first and second trimester of normotensive pregnancies.

Study Design—Circulating levels of maternal placental like growth factor (PLGF), soluble fms-like tyrosine kinase receptor (sFlt1), and soluble endoglin (sEng) were measured in serum samples collected during the first (median 11.3 weeks) and second trimester (median 17.1 weeks) of 182 normotensive pregnancies. Diastolic blood pressure (DBP), systolic blood pressure (SBP), and mean arterial pressure (MAP) were measured at the same visits when samples were collected to measure angiogenic factors. Linear regression analysis was used to examine associations of the angiogenic measures with maternal characteristics. The association between blood pressure measures and concentrations of angiogenic factors was evaluated using Spearman correlation and linear regression analysis.

Results—In adjusted analyses, nulliparous women had higher sFlt1 concentrations in both first ($P=0.06$) and second ($P=0.001$) trimester. Higher BMI was associated with greater sFlt1 concentrations in both the first ($P=0.004$) and second trimester ($P=0.008$), but significantly lower sEng concentrations in both trimesters ($P=0.002$ for first trimester and $P=0.0009$ for second). Nulliparity and higher BMI also were associated with higher sFlt1/PLGF anti-angiogenic ratios in both first ($p=0.05$ and $p=0.007$, respectively) and second trimesters ($p=0.003$ and $p=0.02$, respectively). First trimester sFlt1 levels were weakly correlated with first trimester SBP ($r_s=0.18$, $p=0.03$) and MAP ($r_s=0.16$, $p=0.04$). Second trimester sEng levels were inversely associated with

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second trimester MAP ($r_s = -0.17$, $p=0.05$). Including blood pressure measures in the linear regression models did not change the reported associations of angiogenic factors with maternal characteristics.

Conclusions—These results demonstrate that even early in normotensive pregnancies maternal characteristics are associated with variations in angiogenic profile across this population.

Keywords

Pregnancy; angiogenic factors; sFlt1; PlGF; soluble endoglin; maternal

1. Introduction

During pregnancy, angiogenesis, the process of new blood vessel formation from preexisting vessels, and vascular remodeling are critical in the establishment and functioning of the placenta. The process of angiogenesis during placentation is tightly regulated by both pro- and anti-angiogenic factors that together form an angiogenic balance. In normal pregnancies, the placenta produces high levels of both vascular endothelial growth factor (VEGF) and placental like growth factor (PlGF) [1], pro-angiogenic factors necessary for both vascular remodeling and new vessel formation.

Maternal angiogenic balance and adequate uteroplacental vessel remodeling during placentation is necessary for normal fetal development. In pregnancy complications such as preeclampsia and intrauterine growth restriction (IUGR)/small-for-gestational age (SGA) the maternal angiogenic balance is disturbed [2, 3]. Compared to normal pregnancies, women who develop preeclampsia or SGA have elevated circulating levels of the anti-angiogenic proteins soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) [4–12].

sFlt1, also known as soluble VEGF receptor 1 (sVEGFR-1), binds to VEGF and PlGF and reduces the free concentrations of these proteins in the circulation [13]. Studies of both normotensive and preeclamptic pregnancies have shown that higher ratios of sFlt1 to PlGF levels are associated with blood pressure increases in mid to late pregnancy [14]. Furthermore, in normotensive pregnant women older maternal age was associated with a more anti-angiogenic profile at delivery, including lower PlGF concentrations and a higher sFlt1/PlGF ratio [15]. These combined results have led us to hypothesize that maternal characteristics such as greater BMI, older maternal age, and nulliparity, which also are risk factors for preeclampsia, may be associated with angiogenic balance even in normotensive pregnancies. As cited above, the limited examination of these associations in normotensive pregnancies has involved measurements from late pregnancy or at time of delivery. It is unclear whether these associations could be detected earlier in pregnancy. Here we explore the association of maternal characteristics with angiogenic factors measured in the first and second trimester of pregnancies that remained normotensive through delivery.

2. Materials and Methods

Study population

The study sample is a subset of participants in The Massachusetts General Hospital (MGH) Obstetric Maternal Study (MOMS), a prospective cohort study that recruited pregnant women between 1998–2005 [16]. The initial focus of this study was to examine the association of insulin resistance and inflammation with preeclampsia risk. All women who receive prenatal care at the MGH Obstetric Service and MGH-affiliated community health centers were eligible for inclusion in the MOMS cohort. After informed consent was

obtained, approximately 70 percent of eligible mothers enrolled in the study at the first prenatal visit. During a routine 45-minute interview at their first prenatal visit, women provided health and demographic information which was entered into electronic records. Demographic, medical, and health characteristics of the mothers and babies, such as: race, marital status, maternal age, body mass index, parity, birth weight and blood pressure (including diastolic blood pressure (DBP), systolic blood pressure (SBP) and mean arterial pressure (MAP), measured at the first and second trimester visits), were similar for participants and nonparticipants. Serum samples were collected at the first prenatal visit (typically at 10–12 weeks). Participants in the MOMS study were invited to return to participate in a fasting blood draw and urine collection during the second trimester. The parent study (i.e. MOMS) for the analyses presented here was approved by the MGH Partners Healthcare Committee on Human Research (IRB) (# 1999p-008925) and written, informed consent was obtained from all participants. The proposal for the specific analyses presented here with an anonymized dataset from the parent study was submitted to the NIH Office of Human Subjects Research and was exempted from NIH IRB review.

For this investigation, we obtained a dataset from a subset of the MOMS database (n=9930) who had participated in a second trimester fasting blood sample collection (n=1948). This subset with a second blood collection was similar to the entire study population with regard to parity and BMI, but was slightly older (31.3 vs. 30.2 years) and less ethnically diverse (70% vs. 62% Caucasian). In an effort to evaluate normal, singleton pregnancies, we excluded women who developed preeclampsia (n=35), gestational diabetes mellitus (n=52), and who had multifetal gestations (n=154). In addition, we excluded women with chronic or gestational hypertension (n=484, defined as any blood pressure during pregnancy $\geq 140/90$, regardless of proteinuria). Of the remaining 1223 subjects, 182 had been randomly selected as controls in other studies in which angiogenic proteins were measured [8, 11, 17]. Specifically, change in sFlt-1 concentration from first to second pregnancy has been published from a smaller subset of this population of 182 controls [17]. Other analyses that have included subsets of this control population include comparison of angiogenic factor measurements between normal pregnancies and pregnancies complicated by preeclampsia [8, 11].

The selected 182 women with singleton pregnancies who remained normotensive (defined as BP<140/90) throughout the pregnancy provided a serum sample at their first prenatal visit (ranging from 4.1 to 12.9 weeks, median 11.53 weeks) and a fasting serum sample during the second trimester (ranging from 13.0 to 23.6 weeks, median 17.1 weeks) of the pregnancy.

Laboratory assays

The laboratory assays have been described previously in the reports noted above [8, 11, 17]. Briefly, enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, Minn) were used to assay PlGF, sFlt1, and sEng. The intraassay coefficients of variation (CV) for PLGF, sFlt1 and sEng were 5.6% [17], 3.5% and 3.2% [8], respectively. The interassay CV for PLGF, sFlt1 and sEng were 10.9% [17], 8.1% and 9.5%, respectively [8].

Statistical Analysis

For first trimester analyses, 27 subjects were excluded because the first sample collection occurred during gestational weeks 13–18, which is beyond the first trimester. Anti-angiogenic ratios were calculated by dividing sFlt1 by PlGF or by dividing the sum of sFlt1 and sEng (both in pg/ml) by PlGF. Medians and ranges of maternal angiogenic protein measures in the first and second trimesters, as well as the change from first to second trimester are reported. Maternal age and body mass index (BMI) were analyzed as

categorical variables using tertiles determined by the distribution of age or BMI in the study sample. Parity was a dichotomous variable evaluated as nulliparous or parous. All subjects with available data are included in the analyses of maternal age, BMI, and parity. Linear regression analysis was used to examine the adjusted association of the angiogenic factor measurements with maternal characteristics. Separate models included logarithm-transformed angiogenic factor measurements as the dependent variable. All models included gestational week at blood draw, maternal age, and BMI as continuous variables and parity as a categorical independent variable. Therefore, the concentrations and associations reported are adjusted for these independent variables. In a prior report, angiogenic profile was associated with blood pressure increases from the second to the third trimester of normotensive pregnancies [14]. Here, we assessed whether diastolic blood pressure (DBP), systolic blood pressure (SBP), or mean arterial pressure (MAP), each examined individually, were correlated with angiogenic factors measured in the same trimester. Further, we examined the associations of maternal characteristics with angiogenic factors after adjusting individually for DBP, SBP, or MAP. Analyses were completed with SAS (version 9.0, SAS Institute, Inc., Cary, NC) and statistical significance was defined at two-sided $P < 0.05$.

3. Results

The median age of the women was 32.9 (standard deviation [SD] ± 5.4) years and median body mass index at the first prenatal visit was 25.9 (SD ± 6.0) kg/m². The study population was largely self-identified as Caucasian (n=129) or Hispanic (n=34). Other racial/ethnic groups included Asian (n=6), African-American (n=5), and unknown (n=8). The median length of gestation was 39.7 (SD ± 1.8) weeks (range 26–42.1) and the median birth weight was 3495 g (SD ± 542 ; range 851–4767). The gender of the offspring was 54% male and 46% female.

Medians (and ranges) of the angiogenic factors measured in the first and second trimesters, and the change in the measurements from first to second trimester are reported in Table 1. All angiogenic measures show substantial between-person variation. As expected, PIGF concentrations increased dramatically from first to second trimester, while median sFlt1 and sEng decreased slightly. The increased PIGF and decrease or constant sFlt1 and sEng levels between first and second trimester of pregnancy have been shown in other populations [4, 5], including in a different subgroup of the control population of the MOMS cohort [8]. The increase in PIGF from first to second trimester resulted in much lower anti-angiogenic ratios of sFlt1/PIGF and (sFlt1+sEng)/PIGF in the second trimester compared with the first trimester.

The adjusted associations of maternal characteristics with the angiogenic proteins and ratios measured in the first trimester are presented in Table 2. Maternal age in tertiles demonstrated a nonlinear association with higher PIGF concentrations associated with women in the middle tertile of age 31–34.5 years of age (Table 2, $P=0.03$). Although of borderline significance, nulliparous women had higher first trimester sFlt1 concentrations than parous women ($P=0.06$) resulting in a higher anti-angiogenic sFlt1/PIGF ratio ($P=0.05$). sEng levels were inversely associated with increasing BMI ($P=0.002$) while sFlt1 and sFlt1/PIGF ratio increased with greater BMI ($P=0.004$ and $P=0.007$, respectively).

The associations of the maternal characteristics with second trimester angiogenic factors were largely similar to those found in the first trimester (Table 3) except for maternal age, which no longer demonstrated an association with PIGF. Nulliparous women had higher sFlt1 levels ($P=0.001$) and consequently a higher mean sFlt1/PIGF ratio ($P=0.003$) in the second trimester when compared to parous women. BMI was again inversely associated

with sEng ($P=0.0009$) and positively associated with sFlt1 levels ($P=0.008$). The anti-angiogenic ratio of sFlt1/PlGF also increased with BMI ($P=0.02$).

Systolic (SBP), diastolic (DBP), and mean arterial blood pressure (MAP) were measured at both the first and second trimester visits when samples were collected to measure angiogenic factors. The correlations of SBP, DBP, and MAP with angiogenic factors are presented in Table 4. In the first trimester, sFlt1 levels were weakly correlated with SBP ($r_s=0.18$, $p=0.03$) and MAP ($r_s=0.16$, $p=0.04$). In the second trimester, sEng was weakly inversely correlated with MAP ($r_s=-0.17$, $p=0.05$). Given these results, all analyses presented in Tables 2 and 3 were repeated adjusting for blood pressure measurements, which were within the normal range for all patients. Adding SBP, DBP, or MAP measurements from the appropriate trimester to the analyses did not change the estimates or the significance of the associations reported in Tables 2 and 3 (data not shown). sFlt1 in the first trimester was still weakly associated with first trimester SBP and MAP even when gestational age, and maternal age, BMI, and parity were added to the model, suggesting that the association of sFlt1 with first trimester blood pressure measures was in part independent from these other factors. Conversely, the association of sEng with MAP in second trimester was attenuated when BMI was added to the model.

4. Comment

Few studies have examined the concentrations of angiogenic factors in the first trimester of normotensive pregnancies. Even less explored are the relationships of angiogenic factors measured early in pregnancy with maternal characteristics in normotensive pregnancies. Our data demonstrate significant, independent associations between concentrations of specific angiogenic factors in early, normotensive pregnancies and selected maternal characteristics such as: nulliparity, high BMI, and greater maternal age, which also are risk factors for the development of preeclampsia [18–20]. Our data support previous results from an analysis conducted in a smaller subset of this study population that showed higher sFlt1 levels in nulliparous women [17]. In another population where angiogenic proteins were measured at delivery in normotensive pregnancies, nulliparity also appeared associated with a more anti-angiogenic profile although the results were not statistically significant [15]. In the present study, BMI at first prenatal visit was positively associated with first and second trimester sFlt1 levels and sFlt1/PlGF ratio, while inversely associated with sEng levels at these same time points.

These findings of a direct association of BMI with sFlt1 and sFlt1/PlGF ratio and an inverse association with sEng are novel and differ from prior studies. In a Norwegian cohort, BMI at delivery was not associated with angiogenic profile assessed at delivery in preeclampsia or normotensive pregnancies [15]. A more recent, larger study by Mijal and colleagues found an inverse association of second trimester sFlt-1 concentrations in maternal serum with BMI in 668 normotensive pregnancies [21]. BMI was assessed as a categorical variable in this study using the Centers for Disease Control and Prevention (CDC) BMI guidelines whereas our BMI categories were established using the tertiles from our study population. When our data were reanalyzed using the CDC guidelines, a significant positive association remained between BMI and both first ($p=0.0024$) and second trimester ($p=0.0018$) maternal sFlt-1. Differences between the two study populations that may account for part of this discrepancy include that our study population is smaller ($n=182$), older (76% of the mothers in our study were age 30+ compared to 28% in this prior study), and 98% of our second trimester serum samples were collected before 20 weeks gestation (median 17.1 weeks), whereas in the prior study 96% of the serum samples were collected between 20–28 weeks gestation [21].

The study presented here has some limitations. The samples used for measuring second trimester levels of angiogenic factors were from morning fasting samples, whereas, the participants were not fasting at the time of the first blood collection. However, the patterns of increasing PlGF levels and decreasing or constant sFlt1 and sEng between the first and second trimesters of pregnancy have been shown in other populations [4, 5]. Furthermore, we examined the literature to determine if fasting status has been shown to affect angiogenic factor measurements in human serum and found no publications that have addressed this issue. Future studies could be designed to examine the effect of fasting on concentrations of angiogenic factors. In addition, the factors we assessed such as higher maternal age and maternal BMI could be associated with greater blood pressure measures, and this in turn could explain the noted associations with angiogenic profile. When we adjusted for DBP, SBP, or MAP each individually, the results for the risk factors remained the same with largely unchanged statistical significance. Whereas blood pressure late in pregnancy has been associated with angiogenic profile [14], we have not found evidence of this association in early pregnancy. Possibly, smoking could be a confounder of these findings. Studies of placental explants suggest that exposure to smoke may decrease sFlt1 levels [22], pregnant smokers have lower sFlt1 than non-smokers, and smokers are at a reduced risk for preeclampsia [23, 24]. In the normotensive population presented here, only 11 women were current smokers and data on former smokers were very limited. Therefore, we could not adjust for smoking status in our analyses.

Few studies have evaluated the natural history of angiogenic balance in the beginning of pregnancies that remain uncomplicated, and how it varies with maternal and gestational factors. The present study population is as large as or larger than previous studies focused on circulating levels of hormones or angiogenic proteins in normotensive pregnancies [17, 25]. Here we have shown that in uncomplicated pregnancies, nulliparity, greater maternal age, and higher BMI are associated with a more anti-angiogenic profile in the first and second trimester. It is interesting that these risk factors for preeclampsia, itself an anti-angiogenic state, are also independently associated with a more anti-angiogenic profile in normotensive pregnancies.

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Table 1
Angiogenic Protein Concentrations and Ratios in the First and Second Trimester and Change between Trimesters.

First Trimester	Median	SD	Range
Gestational week at blood draw	11.3	1.5	4.1–12.9
PIGF, pg/ml (n=148)	30.0	22.0	4.7–158.6
sFlt1, pg/ml (n=151)	2123.8	1518.7	339.0–7380.2
sEng, ng/ml (n=113)	6.1	2.0	1.9–13.0
sFlt1/PIGF (n=148)	68.3	73.4	8.7–372.4
(sFlt1+sEng)/PIGF (n=110)	316.1	257.1	44.1–1610.2
Second Trimester			
Gestational week at blood draw	17.1	1.7	13.0–23.6
PIGF, pg/ml (n=182)	116.9	85.1	9.5–653.1
sFlt1, pg/ml (n=182)	2099.5	1900.2	305.5–11231.1
sEng, ng/ml (n=133)	5.0	1.4	2.5–10.6
sFlt1/PIGF (n=182)	16.1	27.1	0.9–248.9
(sFlt1+sEng)/PIGF (n=133)	63.3	56.6	17.9–477.9
Change from 1st to 2nd Trimester			
Weeks between 1 st & 2 nd blood draw	6.0	2.0	2–12
PIGF, pg/ml (n=148)	83.9	62.1	–8.8 to 314.5
sFlt1, pg/ml (n=151)	–167.2	1655.9	–4258.6 to 5500.1
sEng, ng/ml (n=113)	–1.1	2.1	–7.5 to 3.7
sFlt1/PIGF (n=148)	–44.7	65.9	–326.4 to 91.2
(sFlt1+sEng)/PIGF (n=110)	–238.7	234.5	–1204.7 to –9.4

Table 2
Adjusted Mean First Trimester Angiogenic Protein Concentrations by Maternal Characteristics^a.

	PIGF		sFlt1		sEng		sFlt1/PIGF		(sFlt1+sEng)/PIGF	
	mean (n)	P	mean (n)	P	mean (n)	P	mean (n)	P	mean (n)	P
Maternal age at delivery (years)										
≥30.9	29 (49)		1882 (50)		6.5 (41)		65 (49)		330 (40)	
31.0–34.5	33 (49)		2345 (50)		6.5 (37)		70 (49)		284 (36)	
34.6+	26 (50)	0.03	1920 (51)	0.19	5.8 (35)	0.21	74 (50)	0.66	351 (34)	0.20
Parity										
0	29 (82)		2276 (83)		6.2 (63)		78 (82)		330 (62)	
≥1	29 (66)	0.97	1826 (68)	0.06	6.4 (50)	0.61	62 (66)	0.05	308 (48)	0.51
BMI at first trimester collection										
≤24.3	29 (50)		1588 (51)		7.5 (26)		54 (50)		384 (25)	
24.4–28.7	31 (51)		2368 (51)		6.3 (42)		75 (51)		302 (42)	
28.8+	27 (47)	0.25	2253 (49)	0.004	5.6 (45)	0.002	83 (47)	0.007	305 (43)	0.13

^aCategories of age and BMI are based on tertiles. Estimates are from linear regression models with logarithm-transformed angiogenic measure as the dependent variable and maternal age, BMI, gestational age at blood draw and parity as independent variables. Adjusted means are geometric (transformed back from the logarithmic scale).

Table 3
Adjusted Mean Second Trimester Angiogenic Protein Concentrations by Maternal Characteristics^a.

	PIGF		sFlt1		sEng		sFlt1/PIGF		(sFlt1+sEng)/PIGF	
	mean (n)	P	mean (n)	P	mean (n)	P	mean (n)	P	mean (n)	P
Maternal age at delivery (years)										
≥30.9	122 (60)		1799 (60)		5.0 (48)		15 (60)		64 (48)	
31.0–34.6	125 (61)		2001 (61)		5.2 (44)		16 (61)		60 (44)	
34.7+	115 (61)	0.56	1904 (61)	0.75	4.9 (41)	0.50	17 (61)	0.72	63 (41)	0.83
Parity										
0	121 (97)		2293 (97)		5.1 (70)		19 (97)		64 (70)	
≥1	120 (85)	0.86	1573 (85)	0.001	5.0 (63)	0.74	13 (85)	0.003	60 (63)	0.51
BMI at first trimester collection										
≤23.8	118 (61)		1492 (61)		5.5 (34)		13 (61)		64 (34)	
23.9–28.5	126 (60)		2036 (60)		5.3 (44)		16 (60)		63 (44)	
28.6+	118 (61)	0.68	2257 (61)	0.008	4.6 (55)	0.0009	19 (61)	0.02	60 (55)	0.88

^aCategories for age and BMI are based on tertiles. Estimates are from linear regression models with logarithm-transformed angiogenic measure as the dependent variable and maternal age, BMI, gestational age at blood draw and parity as independent variables. Adjusted means are geometric (transformed back from the logarithmic scale).

Spearman correlations (r_s) between blood pressure measures and maternal angiogenic factors measured in the same trimester in women who remained normotensive throughout pregnancy.

Table 4

	First trimester angiogenic factor X first trimester bp				Second trimester angiogenic factor X second trimester bp			
	r_s		p value		r_s		p value	
	SBP	DBP	MAP		SBP	DBP	MAP	
PIGF	0.09 0.29	0.06 0.49	0.07 0.41		0.03 0.68	-0.004 0.96	-0.003 0.96	
sFlt1	0.18 0.03	0.09 0.29	0.16 0.04		0.07 0.33	-0.02 0.77	0.01 0.90	
sFlt1/PIGF	0.09 0.27	0.003 0.97	0.08 0.36		0.02 0.75	-0.02 0.83	-0.002 0.98	
sEng	-0.16 0.09	-0.03 0.74	-0.12 0.22		-0.12 0.16	-0.17 0.06	-0.17 0.05	
(sFlt1+sEng)/PIGF	-0.16 0.10	-0.05 0.63	-0.07 0.48		-0.08 0.38	-0.03 0.76	-0.05 0.60	