Preterm Delivery as a Unique Pathophysiologic State Characterized by Maternal Soluble FMS-Like Tyrosine Kinase I and Uterine Artery Resistance During Pregnancy: A Longitudinal Cohort Study

Reproductive Sciences
2017, Vol. 24(12) 1583-1589
© The Author(s) 2017
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719117698574
journals.sagepub.com/home/rsx

Jennifer K. Straughen, PhD¹, Dawn P. Misra, PhD²,
Laura Helmkamp, MS², and Vinod K. Misra, MD, PhD³

Abstract

Background: Preterm delivery (PTD) may be characterized by altered interrelationships among angiogenic factors and measures of placental function. We analyzed the longitudinal relationship between maternal serum concentrations of soluble fms-like tyrosine kinase I (sFltI), an important antiangiogenic factor, and uterine artery resistance in pregnancies resulting in preterm and term deliveries. **Methods:** Data were collected in a longitudinal cohort study involving 278 women monitored at 6 to 10, 10 to 14, 16 to 20, 22 to 26, and 32 to 36 weeks of gestation. Concentrations of maternal serum sFltI were determined using solid-phase enzyme-linked immunosorbent assay, and uterine artery resistance indices (RI) were measured by Doppler velocimetry at each interval. Preterm delivery was defined as birth before 37-weeks completed gestation. Data analyses used multivariable repeated measures regression models. **Results:** Uterine artery RI decreased across gestation. As pregnancy progressed, RI trajectories diverged for term and preterm deliveries; the mean RI was significantly higher in third trimester for pregnancies resulting in PTD ($P = .08$). sFltI was stable through 21 3/7 weeks of gestation and then increased rapidly; women who delivered preterm had significantly higher sFltI levels in the third trimester ($P = .04$). The relationship between uterine artery RI and sFltI from the prior visit was significantly different between the groups ($P < .0001$). For term deliveries, higher sFltI concentrations were associated with a smaller RI at the subsequent visit ($\beta = -.08$, 95% confidence interval [CI]: -0.14 to -0.02). For PTD, higher sFltI concentrations were associated with a larger uterine artery RI ($\beta = .14$, 95% CI: 0.06 to 0.22). **Conclusion:** PTD is characterized by altered relationships between angiogenic factors and placental vascular blood flow starting in early pregnancy.

Keywords

angiogenic factors, FltI protein, longitudinal analysis, Doppler ultrasound, pregnancy, preterm birth, placental development

Background

The birth of a healthy infant at term depends on the establishment of the maternal–fetal vascular interface early in gestation and ongoing placental development during pregnancy.¹ Disruption of important angiogenic and vasculogenic processes underlying placentation contributes to the pathophysiology of several adverse perinatal outcomes including risk of preterm delivery (PTD).^{2–4} Accordingly, spontaneous preterm delivery is associated with histopathological evidence of disordered placentation^{5–10} and altered placental blood flow.¹¹

Pregnancies that result in PTD may be the result of a distinct pathophysiological state arising from a number of clinical conditions that disrupt the physiological systems involved in placentation and placental vascular development. This state is characterized by dysregulation of factors involved in angiogenesis,^{12–14} a greater degree of failure of physiologic

transformation of the spiral arteries in the myometria and decidua of the placental bed than normal pregnant women at term,⁸ and higher uterine and umbilical artery resistances.¹¹ Since PTD may be a disordered physiologic state starting very early in pregnancy, the interrelationships among angiogenic

¹ Department of Public Health Sciences, Henry Ford Health System, Detroit, MI, USA

² Department of Family Medicine and Public Health Sciences, The Wayne State University School of Medicine, Detroit, MI, USA

³ Department of Pediatrics, Division of Genetic, Genomic, and Metabolic Disorders, The Wayne State University School of Medicine, Detroit, MI, USA

Corresponding Author:

Vinod K. Misra, Division of Genetic, Genomic, and Metabolic Disorders, Children's Hospital of Michigan, 3950 Beaubien Blvd, Detroit, MI 48201, USA. Email: vmisra@med.wayne.edu

factors, antiangiogenic factors, and measures of placental function may be very different in pregnancies that result in PTD when compared to those that result in term deliveries.

Soluble fms-like tyrosine kinase 1 (sFlt1), a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt1, is a potent antiangiogenic protein that antagonizes the effects of the proangiogenic proteins placental growth factor (PLGF) and VEGF. Variation in maternal serum concentrations sFlt1 has been associated with disorders of placental angiogenesis^{12,13} and risk of PTD.¹⁵ Variation in uterine artery resistance as measured by Doppler velocimetry provides functional insight into placental vascular development and blood flow and is itself associated with risk of PTD.^{11,16} We investigate the hypothesis that the relationship between maternal serum concentrations of sFlt1 and uterine artery resistance differs across gestation between pregnancies that result in term and preterm deliveries.

Methods

Data Collection and Study Variables

Data for this study were collected as part of a prospective cohort study of pregnant women who presented for early prenatal care at the University of Michigan Health System (UMHS). Women were eligible for inclusion if they were 18 to 45 years of age, 6 to 10 weeks of gestation at the time of presentation, had a singleton pregnancy, and intended to deliver at the study hospital. The study was approved by the UMHS Institutional Review Board.

Data and laboratory samples were collected at 5 time intervals during pregnancy: 6 to 10, 10 to 14, 16 to 20, 22 to 26, and 32 to 36 weeks of gestation. At each interval, data were obtained from a brief interview, fetal ultrasound measurements, and a maternal blood draw. Additional data such as date of delivery, type of delivery, birth weight, and infant gender were collected at delivery. Data were analyzed for a cohort of 278 participants who delivered a live infant and were measured for all relevant variables during the first 2 years of the study. Participants with major structural fetal abnormalities or prenatally diagnosed aneuploidy were excluded from the analysis. Fewer than 3% of data were missing for any variable.

Gestational age at each visit and delivery were calculated from ultrasound measures of gestational age between 6 and 10 weeks of gestation. Preterm delivery was defined as birth before 37-weeks completed gestation. Pregnancy-induced hypertension was defined using the International Society for the Study of Hypertension in Pregnancy classification.¹⁷

At each visit, maternal blood samples were collected in a serum separator tube and allowed to clot for 30 minutes before centrifugation for 15 minutes at $1000 \times g$. The serum samples were aliquoted and stored -86°C until analysis. Serum concentrations of maternal sFlt1 were determined using solid-phase enzyme-linked immunosorbent assay (ELISA) conducted in duplicate using commercially available kits (R&D Systems, Inc, Minneapolis, Minnesota). Assays were

performed by personnel who were blinded to all patient identifiers and pregnancy outcomes.

At each visit, Doppler measurements on the left and right uterine arteries were obtained just proximal to the point where they crossed the internal iliac artery. These data were collected by 1 of 5 sonographers at a single site trained by and under the supervision of a project coinvestigator solely for research purposes and were not available to clinicians involved in the participants' prenatal care. Sonographers performing research sonograms were blinded to clinical information about the participants.

For each Doppler waveform, the peak systolic velocity (S), end diastolic velocity (D), resistance index ($\text{RI} = (\text{S} - \text{D}) / \text{S}$), and pulsatility index were measured 3 times. Since there may be laterality differences in uterine artery RI, a nested 1-way analysis of variance was used to test the average difference between the left and right uterine artery RI. Since there were no significant differences ($P > .10$) between the average measurements on the 2 sides, the values of the left and right uterine artery RI for each individual were combined, and all 6 measures were averaged together for each visit.

Statistical Analysis

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, North Carolina). Differences between term delivery and PTD were determined using the χ^2 test for categorical variables and the t test for continuous variables. Cross-sectional analyses were performed to compare mean sFlt1 and uterine artery RI values at each of the 5 time intervals for the term and preterm groups, and sFlt1 values were log transformed for normality; then, an independent samples t test was performed at each time interval. For uterine artery RI, no transformation was required before conducting t tests. The risk ratios for preterm delivery were explicitly calculated for sFlt1 and uterine artery RI values at each of the 5 time intervals using a log-binomial model.

Inspection of cross-sectional sFlt1 values indicated that in both the term and the preterm groups, the values remained relatively stable through the fourth visit (22–26 weeks of gestation) and then increased sharply by the fifth visit (32–36 weeks of gestation). The change in $\log(\text{sFlt1})$ over gestation was therefore modeled using a simple piecewise model in which values were allowed to change linearly over time until the 21 3/7 weeks of gestation, at which point a quadratic term was allowed. Model fit was analyzed using both likelihood-based fit statistics (eg, the Akaike information criterion) and an examination of the residual values from the model. This piecewise model had better fit than simple linear and quadratic models, cubic models, and models fit using restricted cubic splines. Multiple points were examined as possible origins of the quadratic increase in mid to late gestation; 21 3/7 weeks was chosen both for its superior model fit and for its simplicity. Qualitative results did not change substantially among these similar models for sFlt1. A repeated measures model with an unstructured

covariance matrix was used to account for correlation between sFlt1 values across visits within a participant.

Inspection of the cross-sectional uterine artery RI values showed that RI decreases over gestation in both the term and the preterm groups, with a faster decrease in early gestation than later. A quadratic model fit this pattern well for both the term and the preterm groups. A repeated measures model with an unstructured covariance matrix was used to account for correlation between uterine artery RI values across visits within a participant; allowing the covariance matrix to differ between term and preterm participants did not improve model fit for uterine artery RI, so nesting within preterm groups was not used for covariance estimation.

The effect of sFlt1 on uterine artery RI was investigated assuming there was a lag in the effect of sFlt1 on uterine artery RI so that the effect of sFlt1 from the prior study visit on the uterine artery RI from the current visit was estimated. The effect of sFlt1 on uterine artery RI was investigated in cross-sectional analyses by fitting a linear model for the uterine artery RI value at that visit on the log(sFlt1) value from the previous visit.

Finally, we use a multivariable repeated measures model to examine whether the effects of sFlt1 from the previous visit on uterine artery RI from the subsequent visit (a “lagged” effect) differ between term and preterm participants. The regression equation used for this model is given by:

$$UTRI = \beta_1 * I[preterm] + \beta_2 * I[term] + \beta_3 * I[preterm] * GA + \beta_4 * I[term] * GA + \beta_5 * I[preterm] * GA^2 + \beta_6 * I[term] * GA^2 + \beta_7 * I[preterm] * lagSFLT + \beta_8 * I[term] * lagSFLT$$

where GA is the gestational age at time t, UTRI is uterine artery RI at time t, and lagSFLT is the maternal serum sFlt1 concentration at time t – 1. This repeated measures model was also fit with an unstructured covariance matrix; the covariance matrix was estimated separately for the term and preterm groups.

Results

Table 1 describes the characteristics of our study sample. Of the 278 pregnancies in our sample, 19 women delivered preterm for an overall PTD rate of 7%. Our sample had a low prevalence of traditional sociodemographic risk factors for PTD. Approximately 81% of our participants were white, 6.1% were African American, and 95.7% were non-Hispanic in origin. Nearly 85.6% our sample were married at the time of enrollment, and 56.5% had achieved at least a college degree; 43.5% had a postgraduate education. Nearly half of our sample reported a total household income of US\$80 000 or greater. More than half (65.1%) of the women in our sample were multiparous. Only 8.9% of our participants smoked during pregnancy. As expected for this relatively homogeneous sample, sociodemographic risk factors, ethnicity, maternal age, parity, marital status, education, income, prepregnancy body mass index, diabetes, and smoking were not significantly associated with risk

Table 1. Characteristics of the Study Sample.

Characteristics	Term Deliveries		Preterm Deliveries		P Value
	n	%	n	%	
Total	259	93.2	19	6.8	
Race					
White	211	81.5	15	79.0	.12 ^a
African American	14	5.4	3	15.8	
Asian	17	6.6	1	5.3	
Other	15	5.8	0	0	
Unknown	2	0.8	0	0	
Ethnicity					>.99
Hispanic	12	4.6	0	0	
Non-Hispanic	247	95.4	19	100	
Maternal age					.48
≤30 years	103	39.8	6	31.6	
>30 years	156	60.2	13	68.4	
Parity					.24
Nulliparous	88	34.0	9	47.4	
Multiparous	171	66.0	10	52.6	
Marital status					.17
Married	224	86.5	14	73.7	
Not married	35	13.5	5	26.3	
Highest educational level completed					.54
College or less	145	56.0	12	63.2	
Postgraduate	114	44.0	7	36.8	
Income					.14
≤US\$80 000 per year	123	47.5	12	63.2	
>US\$80 000 per year	129	49.8	6	31.6	
Missing	7	2.7	1	5.3	
Maternal body mass index, kg/m ²					.84
≤25	130	50.2	10	52.6	
>25	129	49.8	9	47.4	
Diabetes					.66 ^b
Prepregnancy	2	0.8	1	5.3	
Gestational	19	7.3	1	5.3	
No	237	91.5	17	89.5	
Unknown	1	0.4	0	0	
Pregnancy-induced hypertension					.01
Yes	1	0.8	2	10.5	
No	258	99.2	17	89.5	
Smoking					.38
Yes	23	8.9	0	0	
No	230	88.8	19	100.0	
Unknown	6	2.3	0	0	
Mode of delivery					.09
Cesarean section	75	29.0	9	47.4	
Vaginal	184	71.0	10	52.6	
Small for gestational age ^c					>.99
	Mean		Mean		
	(Std. Dev)		(Std. Dev)		
Gestational age at delivery, weeks	39 3/7		34 3/7		<.0001
	(1 1/7)		(3 3/7)		
Birth weight, grams	3480.2		2583.4		<.0001
	(433.9)		(736.5)		

^aP value compares white versus African American; other racial groups have insufficient sample size.

^bP value compares any diabetes versus none.

^cSmall for gestational age (<10th percentile) calculated using sex and gestational age-specific standard.¹⁸

Figure 1. The gestational age dependence of the uterine artery resistance index (RI). Regression lines calculated using a quadratic model are shown for term (solid line) and preterm (dashed line) deliveries.

of PTD. For the 19 cases of PTD, the mean gestational was 34 3/7 ($\pm$ 3 3/7) weeks. Among these cases, 15 were late PTDs (gestational age 34 0/7 weeks to 36 6/7 weeks). There was a low prevalence of chronic disease and pregnancy-related complications, including gestational diabetes, in this low-risk cohort. There were only 4 cases of pregnancy-induced hypertension in the entire cohort, and only 2 were among the preterm deliveries. Excluding these cases from the analyses did not change the results.

We first modeled the trajectories of uterine artery RI and sFlt1 across pregnancy. Figure 1 contains plots of the uterine artery RI trajectories for women who delivered term and preterm. The RI decreases across gestation for all pregnancies. However, the trajectories of the RI for term and preterm deliveries appear to diverge as pregnancy progresses so that the mean uterine artery RI measured during third trimester (32–36 weeks of gestation) was significantly larger for women who delivered preterm ($P = .08$) and predicted a significantly higher risk of PTD ($P = .0017$). Figure 2 contains plots of maternal serum sFlt1 concentrations across pregnancy. In both groups, maternal serum sFlt1 concentrations are relatively constant through early second trimester (21 3/7 weeks of gestation) and then increase rapidly during late second and third trimester such that the mean sFlt1 level was significantly larger in the third trimester (32–36 weeks of gestation) for women who delivered preterm ($P = .04$).

We then tested the hypothesis that the relationship between maternal serum concentrations of sFlt1 and uterine artery RI differs between pregnancies that result in preterm compared to term deliveries. Specifically, the effect of sFlt1 on uterine artery RI was investigated assuming there was a lag in the effect of sFlt1 on uterine artery RI so that the effect of sFlt1 from the prior study visit on the uterine artery RI from the current visit was estimated. This model assumes that variation in sFlt1 levels in early gestation is associated with a pathophysiologic process that influences variation in uterine artery RI

Figure 2. The gestational age dependence of the maternal serum soluble fms-like tyrosine kinase 1 (sFlt1) concentration (ng/mL). Regression lines for term (solid line) and preterm (dashed line) deliveries were calculated using a simple piecewise model, in which values were allowed to change linearly over time until 21 3/7 weeks of gestation, at which point a quadratic term was allowed.

several weeks later. For mothers who delivered term, higher sFlt1 concentrations were associated with a smaller uterine artery RI at the subsequent visit ($\beta = -.08$, 95% confidence interval [CI]: -0.13 to -0.03 ; $P = .0009$), whereas for mothers who delivered preterm higher sFlt1 concentrations were associated with a larger uterine artery RI at the subsequent visit ($\beta = .14$, 95% CI: 0.06 to 0.22 ; $P = .003$). The relationship between sFlt1 and uterine artery RI is shown in Figure 3. The difference in the effect of sFlt1 on the uterine artery RI trajectories between mothers who delivered term and preterm was significant ($P < .0001$). Analyses modeling the relationship between sFlt1 and uterine artery RI contemporaneously (without a lagged effect) were qualitatively similar with inflated, but significant, P values.

Discussion

We hypothesized that pregnancies resulting in PTD may be associated with a unique pathophysiological state that alters the interrelationships among angiogenic factors, antiangiogenic factors, and measures of placental function when compared to pregnancies that result in term deliveries. We investigated this hypothesis in a small but uniquely characterized longitudinal cohort with repeated measures sFlt1 and uterine artery resistance across pregnancy. Our results provide preliminary evidence that the nature of the physiologic relationships among factors involved in placentation may be qualitatively altered from early in gestation in pregnancies resulting in PTD.

As in prior studies,^{11,19–21} our data show that uterine artery RI decreases across gestation for pregnancies that result in both term delivery and PTD with a consistently larger RI starting in early second trimester for pregnancies that deliver preterm

Figure 3. The relationship between maternal serum soluble fms-like tyrosine kinase 1 (sFlt1) concentration (ng/mL) and uterine artery resistance index (RI) plotted at median gestational age (GA) of 19 2/7 weeks for term (solid line) and preterm (dashed line) deliveries. The relationship between sFlt1 and uterine artery RI does not vary with GA, although the predicted values of uterine artery RI do change with GA.

(Figure 1). The observed trends in the uterine artery RI support the hypothesis that preterm pregnancies may be associated with abnormalities in processes needed to transform the uteroplacental vasculature into a low-resistance, high-capacity system as pregnancy progresses.^{1,2,4,13,22-24} Maternal serum sFlt1 concentrations are relatively constant through early second trimester (about 21 3/7 weeks of gestation) and then increase rapidly for both term and preterm pregnancies. However, for mothers who delivered preterm, sFlt1 concentrations tended to be higher starting in the first trimester and increased very rapidly in second and third trimesters (Figure 2). Along with these differences, our analyses show that pregnancies resulting in PTD are characterized by a significantly different longitudinal relationship between maternal serum concentrations of sFlt1 and uterine artery resistance compared to pregnancies that resulted in term deliveries. For mothers who delivered term, higher maternal serum sFlt1 concentrations were associated with a smaller uterine artery RI later in pregnancy, whereas for mothers who delivered preterm, higher maternal serum sFlt1 concentrations were associated with a larger uterine artery RI later in pregnancy. These differences may reflect a disorder of placentation starting in early gestation.

Placentation involves extensive angiogenesis in maternal and fetal placental tissues accompanied by a marked increase in placental blood flow as pregnancy progresses.²⁵ This process requires the precise spatiotemporal coordination of many angiogenic factors, including VEGF, PLGF, and their cognate protein tyrosine kinase receptors, including Flt1 and Flk1,^{1,2,4,13} among other factors. Accordingly, both VEGF and PLGF can be detected in maternal plasma in early gestation and rise to a peak at the end of the first trimester.^{26,27} These

factors are associated with the process that results in increased placental blood flow as pregnancy progresses.

The soluble receptor sFlt1 is an antiangiogenic factor secreted primarily by syncytiotrophoblasts into the maternal circulation.¹³ It antagonizes the effects of PLGF and VEGF by sequestering circulating ligand or dimerizing with full-length receptor and preventing signal transduction. Increases in VEGF and PLGF activity during normal pregnancies result in a concomitant rise in circulating sFlt1 levels, presumably to compensate and control elevated systemic bioactivity of these proangiogenic factors during pregnancy.²⁷ In healthy pregnancies that result in term delivery, the maternal sFlt1 level is likely to reflect its normal physiologic role as an antiangiogenic factor that modulates VEGF and PLGF activity so that higher sFlt1 concentrations mirror increases in VEGF activity that are associated with a smaller uterine artery RI later in pregnancy.

In contrast, placentation in pregnancies resulting in PTD may be a disordered physiologic state that leads to aberrant blood flow in the placenta with local tissue hypoperfusion and hypoxia. This phenomenon underlies the relationship of abnormal fetal growth to preterm delivery.²⁸ Prior work has shown that placentas from pregnancies complicated by PTD are found to have a greater degree of failure of physiologic transformation of the spiral arteries in the myometria and decidua of the placental bed than in normal pregnant women at term.⁵⁻⁸ Accordingly, early elevations in maternal serum sFlt1 levels seen in pregnancies that deliver preterm (Figure 2) may be a response to upregulated VEGF expression accompanying impaired cytotrophoblast invasion of the uterine blood vessels and resulting tissue hypoxia early in pregnancy.²⁹⁻³²

These abnormalities may result in compromised placental blood flow in deliveries that result in PTD. Thus, the rate at which the uterine artery RI decreases is smaller for pregnancies that result in PTD.¹¹ Using a lagged model, we suggest that abnormalities of placentation early in pregnancy fundamentally alter the relationship between angiogenic factors, placental angiogenesis, and fetoplacental blood flow that ultimately manifests in reduced uterine artery flow. Aberrant blood flow may lead to tissue hypoxia that progressively upregulates expression of proangiogenic factors, such as VEGF, to facilitate angiogenesis in the compromised placenta.²⁹⁻³² Increased levels of these proangiogenic factors may be a primary compensatory mechanism to the underlying pathophysiologic process as pregnancy progresses. Concomitant increases in placental sFlt1 expression may accompany such changes,³³ with the largest increases occurring late in pregnancy. Such effects are seen in placentas from pregnancies complicated by severe preeclampsia with IUGR.^{30,34,35} As a result, in pathologic pregnancies that result in preterm delivery, maternal sFlt1 concentrations may be a reflection of the disordered physiologic state that results in markedly elevated expression of proangiogenic factors (eg, VEGF) in the placenta so that higher maternal serum sFlt1 levels mirror these levels and are associated with a larger uterine artery RI.

Although we present a model for disordered placentation in pregnancies resulting in PTD that explains the abnormal

relationship between maternal sFlt1 concentrations and uterine artery RI, our data do not distinguish whether increased placental sFlt1 production is a primary or secondary event. In our analysis, we examined the effects of sFlt1 from the prior study visit on the uterine artery RI from the current visit which strongly suggests that changes in sFlt1 concentration are an antecedent effect. Nevertheless, a variety of clinical conditions including acute infection, chronic infection, abnormal inflammatory responses, altered homocysteine metabolism, dyslipidemia, and insulin resistance can influence placental vascular development. Thus, abnormal placentation may mediate the effects of a range of environmental, genetic, and physiological factors on the risk of PTD. If aberrant angiogenesis, perhaps a consequence of inflammatory or infectious factors, is an early event in PTD, increased sFlt1 release may be a secondary phenomenon. That is to say, abnormal placental vascular development may represent a final common pathway for a number of pathologic processes that are associated with PTD.³ However, it remains possible that primary dysregulation of angiogenic factors results in aberrant angiogenesis giving rise to abnormal uterine artery blood flow.

Our study is among the first to demonstrate an abnormal relationship between maternal serum sFlt1 concentration and placental blood flow in pregnancies affected by PTD; our analyses are limited by the sample size. The prospective cohort design of this study is ideally suited for measuring longitudinal data for the analysis of time dependence of key variables over the course of gestation. Moreover, this design also ensures that an accurate estimate of gestational age can be determined from an early ultrasound examination. However, the identification and recruitment of study participants early in pregnancy inherently limits study participation to a group of women who present for prenatal care and are able to attend multiple study visits. Women without prenatal care or with late, interrupted, or sporadic care are less likely to have been included. As a result, our sample size is relatively small and low risk with a preterm delivery rate of 6.8%.

The statistical models (ie, the linear mixed model) used here specifically addresses time-dependent exposures and outcomes. Measurement of variables starting in first trimester provides a unique and comprehensive view of the dynamics of the relationship between sFlt1, uterine artery RI, and risk of PTD across the entire span of gestation. Moreover, in contrast to most prior studies, we have used a repeated measures analysis appropriate for longitudinal data. This analysis enhances the precision of the parameter estimates and increases the power to detect differences.³⁶ Thus, despite the relatively small number of preterm deliveries, our results are likely robust. Larger studies that can more thoroughly model the temporal relationship between various angiogenic factors and placental blood flow will be needed to distinguish between the different pathogenic possibilities described earlier. In addition, the interplay among various proangiogenic and antiangiogenic factors will have to be considered more fully in larger longitudinal studies to appreciate the role of this system in the etiology of PTD.

Conclusions

Our findings have important implications for understanding the pathogenesis of PTD. Our results suggest that pregnancies resulting in PTD may be the result of a distinct pathophysiological state that alters the fundamental interrelationships among angiogenic factors and their relationship with placental function, rather than a consequence of a supraphysiologic normal response. A better understanding of interrelationships among angiogenic factors, their relationship with normal and abnormal placental angiogenesis, and pregnancy outcomes will ultimately lead to improved management of pregnancies that may be affected by PTD. Moreover, as a potential etiologic mechanism, placental vascular dysfunction may offer novel preventative and therapeutic possibilities for some pregnancies at risk of PTD.

Authors' Note

Written informed consent was obtained from all participants prior to enrollment in the study. The study was approved by the University of Michigan Health System Institutional Review Board (IRBMED #00000540).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: VKM was supported by a Doris Duke Clinical Scientist Development Award (Grant 2007092) and a National Institutes of Health Mentored Scientist Award (K08-HD045609). The Michigan Clinical Research Unit which was supported by a Clinical and Translational Science Award grant number UL1RR024986 from the National Institutes of Health. JKS was supported by a postdoctoral award from Wayne State University.

References

1. Regnault TR, Galan HL, Parker TA, Anthony RV. Placental development in normal and compromised pregnancies—a review. *Placenta*. 2002;23(suppl A):S119-S129.
2. Kingdom J, Huppertz B, Seaward G, Kaufmann P. Development of the placental villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol*. 2000;92(1):35-43.
3. Thorp JM. Placental Vascular Compromise: unifying the etiologic pathways of perinatal compromise. *Curr Probl Obstet Gynecol Fertil*. 2001;24(6):203-220.
4. Torry DS, Hinrichs M, Torry RJ. Determinants of placental vascularity. *Am J Reprod Immunol*. 2004;51(4):257-268.
5. Arias F, Rodriquez L, Rayne SC, Kraus FT. Maternal placental vasculopathy and infection: two distinct subgroups among patients with preterm labor and preterm ruptured membranes. *Am J Obstet Gynecol*. 1993;168(2):585-591.
6. Arias F, Victoria A, Cho K, Kraus F. Placental histology and clinical characteristics of patients with preterm premature rupture of membranes. *Obstet Gynecol*. 1997;89(2):265-271.

7. Kim YM, Chaiworapongsa T, Gomez R, et al. Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. *Am J Obstet Gynecol*. 2002;187(5):1137-1142.
8. Kim YM, Bujold E, Chaiworapongsa T, et al. Failure of physiologic transformation of the spiral arteries in patients with preterm labor and intact membranes. *Am J Obstet Gynecol*. 2003;189(4):1063-1069.
9. Faye-Petersen OM. The placenta in preterm birth. *J Clin Pathol*. 2008;61(12):1261-1275.
10. Salafia CM, Ernst LM, Pezzullo JC, Wolf EJ, Rosenkrantz TS, Vintzileos AM. The very low birthweight infant: maternal complications leading to preterm birth, placental lesions, and intrauterine growth. *Am J Perinatol*. 1995;12(2):106-110.
11. Misra VK, Hobel CJ, Sing CF. Placental blood flow and the risk of preterm delivery. *Placenta*. 2009;30(7):619-624.
12. Wang A, Rana S, Karumanchi SA. Preeclampsia: the role of angiogenic factors in its pathogenesis. *Physiology (Bethesda)*. 2009;24:147-158.
13. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and Angiogenic Imbalance. *Ann Rev Med*. 2008;59(1):61-78.
14. Straughen JK, Misra DP, Kumar P, Misra VK. The influence of overweight and obesity on maternal soluble fms-like tyrosine kinase 1 and its relationship with leptin during pregnancy. *Reprod Sci*. 2013;20(3):269-275.
15. Straughen JK, Kumar P, Misra VK. The effect of maternal soluble FMS-like tyrosine kinase 1 during pregnancy on risk of preterm delivery. *J Matern Fetal Neonatal Med*. 2012;25(10):1879-1883.
16. Gagnon R, Van den Hof M; Diagnostic Imaging Committee, Executive and Council of the Society of Obstetricians and Gynaecologists of Canada. The use of fetal Doppler in obstetrics. *J Obstet Gynaecol Can*. 2003;25(7):601-614; quiz 615-606.
17. Tranquilli AL, Dekker G, Magee L, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens*. 2014;4(2):97-104.
18. Talge NM. *Pediatrics* 2014;133(5):844-853.
19. Strigini FA, Lencioni G, De Luca G, Lombardo M, Bianchi F, Genazzani AR. Uterine artery velocimetry and spontaneous preterm delivery. *Obstet Gynecol*. 1995;85(3):374-377.
20. Fonseca E, Yu CK, Singh M, Papageorgiou AT, Nicolaides KH. Relationship between second-trimester uterine artery Doppler and spontaneous early preterm delivery. *Ultrasound Obstet Gynecol*. 2006;27(3):301-305.
21. Cobian-Sanchez F, Prefumo F, Bhide A, Thilaganathan B. Second-trimester uterine artery Doppler and spontaneous preterm delivery. *Ultrasound Obstet Gynecol*. 2004;24(4):435-439.
22. Caniggia I, Winter J, Lye SJ, Post M. Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta*. 2000;21(suppl A):S25-S30.
23. Patel J, Landers K, Mortimer RH, Richard K. Regulation of hypoxia inducible factors (HIF) in hypoxia and normoxia during placental development. *Placenta*. 2010;31(11):951-957.
24. Kaufmann P, Mayhew TM, Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. *Placenta*. 2004;25(2-3):114-126.
25. Reynolds LP, Redmer DA. Angiogenesis in the placenta. *Biol Reprod*. 2001;64(4):1033-1040.
26. Evans PW, Wheeler T, Anthony FW, Osmond C. A longitudinal study of maternal serum vascular endothelial growth factor in early pregnancy. *Hum Reprod*. 1998;13(4):1057-1062.
27. Levine RJ, Maynard SE, Qian C, et al. Circulating Angiogenic Factors and the Risk of Preeclampsia. *N Engl J Med*. 2004;350(7):672-683.
28. Zeitlin J, Ancel PY, Saurel-Cubizolles MJ, Papiernik E. The relationship between intrauterine growth restriction and preterm delivery: an empirical approach using data from a European case-control study. *BJOG*. 2000;107(6):750-758.
29. Rana S, Karumanchi SA, Levine RJ, et al. Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. *Hypertension*. 2007;50(1):137-142.
30. Zhou Y, McMaster M, Woo K, et al. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol*. 2002;160(4):1405-1423.
31. Smith GC, Crossley JA, Aitken DA, et al. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. *Obstet Gynecol*. 2007;109(6):1316-1324.
32. Harris LK. IFPA Gabor Than Award lecture: Transformation of the spiral arteries in human pregnancy: key events in the remodeling timeline. *Placenta*. 2011;32(suppl 2):S154-S158.
33. Hornig C, Barleon B, Ahmad S, Vuorela P, Ahmed A, Weich HA. Release and complex formation of soluble VEGFR-1 from endothelial cells and biological fluids. *Lab Invest*. 2000;80(4):443-454.
34. Geva E, Ginzinger DG, Zaloudek CJ, Moore DH, Byrne A, Jaffe RB. Human placental vascular development: vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2. *J Clin Endocrinol Metab*. 2002;87(9):4213-4224.
35. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003;111(5):649-658.
36. Vickers AJ. How many repeated measures in repeated measures designs? Statistical issues for comparative trials. *BMC Med Res Methodol*. 2003;3:22.