Published in final edited form as:

Pregnancy Hypertens. 2012 April 1; 2(2): 158–163. doi:10.1016/j.preghy.2012.01.005.

Second trimester anti-angiogenic proteins and preeclampsia

Catherine L. Haggerty, Ph.D., M.P.H.¹, Michael E. Seifert, M.D.², Gong Tang, Ph.D.³, Jorn Olsen, MD, Ph.D.^{4,5}, Debra C. Bass, M.S.¹, S. Ananth Karumanchi, M.D.⁶, and Roberta B. Ness, M.D., M.P.H.⁷

¹Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA

²Children's Hospital, Harvard Medical School, Boston, MA

³Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA

⁴Department of Epidemiology, School of Public Health, UCLA, Los Angeles, CA

⁵University of Aarhus, Denmark School of Public Health and the Danish Epidemiology Science Centre, Aarhus, Denmark

⁶Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

⁷University of Texas School of Public Health, Houston, TX

Abstract

Objective—To measure the relationships between soluble fms-like tyrosine kinase-1 (sFlt1), soluble endoglin (sEng) and preeclampsia.

Study design—We utilized a nested case-control study comprised of 211 preeclamptic women and 213 normotensive women with primiparous singleton pregnancies enrolled from 13 and <27 gestational weeks among the Danish National Birth Cohort of 100,000 women. Relationships between sFlt1, sEng and preeclampsia were estimated using smoothing splines in generalized linear models, adjusting for maternal age, body mass index, pre-existing hypertension, smoking, and gestational age.

Main outcome measures—Preeclampsia was confirmed by an *International Classification of Diseases* (ICD) discharge diagnosis of 637.03, 637.04 637.09, 637.19 (ICD-8) or DO14 to DO15 (ICD-10) in the National Hospital Discharge Registry. In this sample, few cases delivered small for gestational age infants (8.1%) and the mean gestational age at delivery was term (38.2 ± 2.3 weeks).

Results—Doublings in the expressions of sFlt1 and sEng were associated with 39% (95% CI = 3%, 86%) and 74% (95% CI = 1%, 198%) increased risks of preeclampsia respectively.

© 2012 International Society for the Study of Hypertension in Pregnancy. Published by Elsevier B.V. All rights reserved.

For reprint requests, please contact: Catherine L. Haggerty, Ph.D., M.P.H. Associate Professor of Reproductive Epidemiology, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, 516B Parran Hall, Pittsburgh, PA 15261, haggerty@pitt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusions—We conclude that second trimester high sFlt1 and sEng levels were possibly associated with an increased risk of preeclampsia after adjustment for maternal factors traditionally associated with the syndrome.

INTRODUCTION

Preeclampsia is a systemic maternal disease characterized by the onset of hypertension and proteinuria after 20 weeks of gestation¹ and is a cause of considerable perinatal morbidity and mortality worldwide. Although the etiology of preeclampsia is not understood, one hypothesized pathophysiologic model posits two stages in the development of preeclampsia, the first being abnormal placentation and the second being translation to the maternal systemic disorder.¹⁻³

There is evidence to suggest that the maternal syndrome may be initiated after the release of placental factors, such as anti-angiogenic proteins, into the maternal circulation. In particular, soluble fms-like tyrosine kinase-1 (sFlt1), also called vascular endothelial growth factor receptor-1 (VEGF-R1), is an anti-angiogenic protein which is high in the placental tissue, amniotic fluid, and sera of some women with preeclampsia⁴⁻¹⁰ Similarly, elevated levels of soluble endoglin, another anti-angiogenic protein which is a co-receptor for transforming growth factor β 1 and β 3,¹¹ has also been associated with incident preeclampsia.^{11;12} These anti-angiogenic proteins were elevated not only during clinical disease but also 5 to 8 weeks before the onset of clinical signs and symptoms.¹¹

The placenta is believed to be the source of increased anti-angiogenic proteins in pregnancy, and accordingly the association between these factors and preeclampsia grows stronger after the first trimester.^{12;13} In this investigation, we evaluated independent associations between sFlt1 or sEng and preeclampsia by gestational age in the second trimester. We compared cases of preeclampsia to controls without the disease within the Danish National Birth Cohort,¹⁴ grouping protein concentrations by the gestational age at which they were obtained.

METHODS

Study Population

We performed a nested case-control study examining the relationships between anti-angiogenic factors and preeclampsia among the Danish National Birth Cohort (DNBC), a longitudinal population-based study of 101,033 pregnancies and offspring. The DNBC was approved by the Danish Ethics Central Committee, and details regarding recruitment, retention, and data collection have been previously published (www.DNBC.dk).¹⁴ Briefly, from 1996 to 2003, general practitioners recruited women at their first prenatal visit. The first prenatal examination consisted of pregnancy confirmation and blood sampling, and women were interviewed twice during pregnancy and twice after delivery. In our study, data from cohort members were merged with National Birth Register and National Hospital Discharge Register data by means of a unique personal code given to each citizen in Denmark. Multiparous women, women with multiple gestations, and women with preexisting hypertension were excluded from our current substudy, because the available epidemiologic evidence suggests fundamental differences in risk patterns associated with preeclampsia in these women. In particular, multiparous women who develop preeclampsia often have a history of prior preeclampsia and may be more likely to be at subclinical cardiovascular risk.² Women with multiple gestations are also excluded because of their predictably higher risk for developing preeclampsia. We analyzed sFlt1 and sEng among a subset of 575 single gestation primiparous preeclamptic and normotensive pregnancies enrolled from 13 and <27 weeks of gestation (mean 17.3 ± 3.3 weeks). One hundred and

fifty-one records with no specimens or with values of sFlt1 or sEng ($\log(\text{sFlt1}) < 8.5$ or $\log(\text{sEng}) < 11$) outside of the range expected in the second trimester of pregnancy were excluded. There was no difference in maternal characteristics or pregnancy outcomes between those who were included versus those excluded. This resulted in a sample of 211 primiparous, single gestation preeclamptic women and 213 randomly selected primiparous women with normotensive singleton pregnancies. Institutional Review Board approval was obtained from the University of Pittsburgh.

Preeclampsia Definition

Cases and controls were selected among primiparous, single gestation women who completed both a first and second trimester interview. Four hundred seventy cases were identified by a positive report of preeclampsia at the postnatal interview which was confirmed by an *International Classification of Diseases* (ICD) discharge diagnoses of 637.03, 637.04, 637.09, 637.19 (ICD-8) or DO14 to DO15 (ICD-10) in the National Hospital Discharge Registry. A previously published chart abstraction study within the DNBC demonstrated that the Danish National Hospital Discharge Registry yields a highly specific diagnosis of preeclampsia (99%), as compared to chart review using American College of Obstetrics and Gynecology (ACOG) criteria.¹⁵ In this validation study, mild preeclampsia was defined as either systolic or diastolic blood pressure $\geq 140/90$ mmHg measured twice with an interval of at least 6 hours and the presence of proteinuria (≥ 0.3 g/24 hours or 1+ urine dipstick measured twice with an interval of at least 4 hours). Severe preeclampsia was defined when one or more of the following were present: 1) blood pressure $\geq 160/110$ mmHg measured twice with an interval of at least 6 hours; 2) proteinuria ≥ 5.0 g/24 hours of dipstick $\geq 3+$ measured twice with an interval of at least 4 hours; or 3) oliguria, cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or upper right-quadrant pain, impaired liver function, thrombocytopenia, or fetal growth restriction. Cases were further categorized as having severe preeclampsia if an ICD-10 code of O141 or O150–159 was identified in the registry. Preeclampsia resulting in delivery of a small for gestational age infant was considered as an additional outcome, based on the lowest 5th percentile of birthweight by gestational age at delivery and gender. Lastly, preterm preeclampsia was considered using various cut-points. Late preterm preeclampsia was defined as preeclampsia resulting in delivery after 34 weeks but before 37 weeks of gestation, and early preterm preeclampsia was defined as preeclampsia resulting in delivery before 34 weeks of gestation. Pregnancies delivering at 37 and 0/7 weeks of gestation or later were considered term.

Measurement of sFlt1 and sEng

Whole blood specimens were obtained from each woman in the DNBC at the first study visit and were mailed to the Statens Serum Institut in Copenhagen. Upon arrival, blood was separated and stored at -80°C . The time from collection to processing and storage was one day for 79%, two days for 91%, and within five days for 99% of samples. In general, it has been reported that biomarker studies are not compromised when using whole blood samples stored for 28 hours,¹⁶ the average time from collection to processing in our study. For each woman in our substudy, sFlt1 and sEng were subsequently measured from a single stored serum specimen obtained during the first study visit. Concentrations of sFlt1 and sEng were determined by taking the mean of duplicate assays conducted by a single investigator (M.E.S.) masked to case control status using enzyme-linked immunosorbent assay (ELISA) commercial kits (R&D Systems, Minneapolis, MN). Minimum detectable levels for sFlt1 and sEng were 5 pg/mL and 7 pg/mL, respectively. Interassay coefficients were 5% and 17%, respectively.

Maternal Characteristics

Maternal age was measured at baseline. Pre-pregnancy body mass index (BMI) was determined using reported height and weight at the first interview and was categorized as underweight or normal (<25), overweight (≥25 and <30), or obese (≥30). Self-reported smoking status was obtained from the first interview.

Statistical Analysis

Demographic, clinical, and behavioral characteristics were compared between preeclamptic cases and normotensive controls using the chi-square test of proportions. Logistic regression models were used to compute the corresponding odds ratios and 95% confidence intervals. In statistical analyses, the logarithm of sFlt1 and sEng with base 2 were used. A one unit increment in the log scale corresponds to a doubled expression level. Mean and median sFlt1 and sEng were compared using two sample t-tests. The relationships between gestational age and the expression levels of sFlt1 or sEng were explored through multivariate linear models with smoothing splines for gestational age among severe preeclampsics, mild preeclampsics, and controls. Potential confounders such as maternal age, BMI, smoking, and gestational age at serum collection were adjusted for. Subsequently, generalized linear models were used to find out whether sFlt1 or sEng were predictive of the risks of preeclampsia, severe preeclampsia, and early preterm preeclampsia, adjusting for maternal age, BMI, smoking, and gestational age at serum collection. Estimates of the effects of sFlt1 and sEng were modeled by smoothing splines in these models. The functional interaction between sFlt1 or sEng and the gestational age at serum sampling were also explored. All analyses were carried out in R and the GAM and MGCV packages were used in the prediction model for preeclampsia status.¹⁷

RESULTS

Demographic, clinical, and behavioral characteristics of preeclamptic cases and normotensive controls are presented in Table 1. The gestational age at serum collection did not differ between cases and controls. Overweight women were nearly twice as likely (OR 1.7, 95% CI 1.1–2.8) and obese women over six times as likely (OR 6.2, 95% CI 2.6 – 14.4) to develop preeclampsia. Cigarette smokers were 60% less likely to develop preeclampsia (OR 0.4, 95% CI 0.2 – 0.9). Preeclamptic women had a significantly earlier gestational age at delivery and were more likely to deliver a preterm infant.

When measured within second trimester, preeclampsics had higher levels of sFlt1 and sEng, although the unadjusted differences were not statistically significant (see Table 2). sFlt1 levels were predictive of preeclampsia after adjusting for maternal age, BMI, smoking, and gestational age at serum collection ($p=0.02$). A doubling in the expression of sFlt1 was associated with a 39% increased risk of preeclampsia (95% CI = 3.3%, 86.1%). Because sFlt1 was measured at various gestational ages, from week 13 to week 27, we explored the interactions between the gestational age of serum sample and sFlt1 by incorporating their tensor products in the generalized additive model. The interaction term was not significant with a p -value of 0.54. sEng was also predictive of preeclampsia after adjusting for maternal age, BMI, and smoking ($p=0.04$). A doubling in the expression of sEng was associated with a 74% increased risk of preeclampsia (95% CI = 1.4%, 198%). The interaction between sEng and gestational age at serum collection was not significant ($p=0.26$).

In separate analyses, we evaluated whether sFlt1 and sEng were predictive of severe preeclampsia, preterm preeclampsia, early preterm preeclampsia, and preeclampsia with SGA (see Table 2). Both sFlt1 and sEng were higher among women with severe preeclampsia as compared to controls, although difference in sEng was not statistically

significant. There was a trend toward increased sFlt1 among preeclamptics who delivered a small for gestational age baby, and sEng was significantly higher among preeclamptics with an SGA birth as compared to controls (median 4.8 vs. 4.1, log-transformed p-value < 0.0001). In multivariate models, sFlt1 and sEng were trended to predict the risk of severe preeclampsia (p=0.09, 0.13, respectively); sFlt1 and sEng were not predictive of preeclampsia with SGA (p=0.77, 0.16, respectively).

Lastly, in Table 3 we compared median values of sFlt1 and sEng by case control status among women with sera collected between 20 and 27 weeks gestation, in order to examine a smaller gestational age window closer to the time of preeclampsia development. Median sFlt1 was significantly higher among women who subsequently developed preeclampsia (1313.1 pg/mL vs. 1168.1 pg/mL, log-transformed p-value = 0.03), and median sEng was higher among preeclamptics who delivered a small for gestational age infant as compared to controls (4.4 ng/mL vs. 4.2 ng/mL) although this difference was of borderline statistical significance (p=0.09).

DISCUSSION

In our case-control study of primiparous women nested within a longitudinal population-based study of 101,033 pregnancies in Denmark, we found that among women with angiogenic factors measured within the second trimester, women with preeclampsia had modestly higher levels of sFlt1 and sEng than normotensive controls. After adjustment for factors known to influence preeclampsia, both sFlt1 and sEng concentrations were associated with preeclampsia. Although there were trends toward higher levels of sFlt1 and sEng among severe preeclamptics and preeclamptics who delivered a small for gestational age infant, only the relationships between sFlt1 and severe preeclampsia, and between sEng and preeclampsia with SGA were statistically significant.

Our findings are generally consistent with those found in previous studies of second trimester anti-angiogenic factors and preeclampsia.^{4;8;11;12;18-20} In a case-control study nested within the Calcium for Preeclampsia Prevention (CPEP) trial (cohort n=4589), Levine et al noted significantly higher concentrations of sFlt1 between 21 and 32 weeks gestation among severe preeclamptics (n=29) as compared to normotensive controls (n=120).⁴ In this same cohort, the mean serum level of sEng was significantly higher at 17 to 20 weeks of gestation among women who subsequently developed preterm preeclampsia as compared to normotensive controls (10.2 ng/mL vs. 5.8 ng/mL, p<0.0001).¹¹

Elevations were noted later among term preeclamptics as compared to normotensive controls, with sEng significantly higher at 25 through 28 weeks of gestation (8.5 ng/mL vs. 5.9 ng/mL, p<0.0001).¹¹ Similarly, in another nested case-control study of 39 preeclamptics and 147 normotensive controls, Rana et al reported that sFlt1 concentrations were 33% higher and sEng concentrations 23% higher in the second trimester.¹⁸

Our study builds upon these prior investigations by adjusting for risk factors traditionally associated with preeclampsia, and thus our models report adjusted estimates of the effect of anti-angiogenic proteins on preeclampsia, although confounding may still play a role. Additionally, instead of usual linear models or generalized linear models, we utilized generalized linear models with penalized splines, providing us a robust and powerful tool to investigate the association between anti-angiogenic proteins and preeclampsia. A major strength of our study is the large sample size, but results do have limited generalizability, as most women in our study were Caucasian. Further, we did not have data on the gestational age at preeclampsia diagnosis. As a result, we could not verify if angiogenic factors were measured prior to preeclampsia, nor could early onset preeclampsia be considered as an

outcome. As the gestational age of serum collection ranged from 13 and <27 weeks of gestation with a mean of 17 weeks, it is possible that some cases may have had preeclampsia at the time angiogenic factors were measured. Although early onset preeclampsia could not be considered as an outcome, the DNBC did measure the gestational age at delivery, and by categorizing early preterm preeclampsia as a preeclamptic pregnancy delivered before 34 weeks of gestation, we were able to capture women who experienced both early onset and preterm preeclampsia. However, our study had limited samples with severe preeclampsia, early preterm preeclampsia, or preeclampsia with SGA, where the angiogenic markers have been found to have the strongest association. Indeed, we did find a significant association between sEng and preeclampsia with SGA, but this sample of cases was small and some analyses in our study correspondingly had wide confidence intervals. Another limitation of our study was that specimen collection was limited to a single sample obtained in the second trimester and therefore we were unable to analyze specimens taken from patients closer to the disease signs and symptoms. Additionally, we were unable to determine if angiogenic factors were elevated at multiple time points throughout pregnancy, as demonstrated in previous reports.^{12;13} Despite this, we did find a strong relationship between sFlt1 and preeclampsia when angiogenic factors were measured between 20 and 27 weeks of gestation. Finally, limited sample volumes did not permit us to measure placental growth factor (and ratios with other angiogenic factors) which have been shown to be altered quite early in pregnancy destined to develop preeclampsia.^{4;11;19;21–23} Indeed, some investigations have demonstrated that sFlt1 and sEng are not strongly predictive of preeclampsia, in comparison to placental growth factor or ratios of angiogenic factors.^{24;25}

Among the strongest predictors of preeclampsia in our study was elevated BMI. This is in agreement with several lines of evidence suggesting that pregnancy may represent a “stress test” of maternal carbohydrate, lipid, inflammatory pathways, and vascular function,^{26–29} and as such may unmask preexisting cardiovascular disease risk and display it in the form of preeclampsia. Indeed, many clinical factors are common to cardiovascular disease and preeclampsia. These include: older age,³⁰ adiposity,^{26;31} hypertension,³² and family history of heart disease.³³ Paradoxically, cigarette smoking has been found in many studies to be associated with a lower risk for preeclampsia.^{4;34} In accord, women with preeclampsia in our study were 70% less likely to smoke cigarettes than women without preeclampsia. After adjusting for these well established preeclampsia risk factors, both sFlt1 and sEng were associated with of preeclampsia.

CONCLUSION

In summary, sFlt1 and sEng measured between 13 and 27 weeks of gestation were associated with subsequent preeclampsia after adjustment for maternal factors traditionally associated with the syndrome. sEng measured between 20 and 27 weeks of gestation was also associated with preeclampsia resulting in the delivery of a small for gestational age infant, although this only represented a small percentage of cases.

Acknowledgments

Funding Support: R01HD048669 from the National Institute of Allergy and Infectious Diseases

Reference List

1. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. *JAMA*. 2002; 287:3183–3186. [PubMed: 12076198]

2. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *American Journal of Obstetrics & Gynecology*. 1996; 175:1365–1370. [PubMed: 8942516]
3. Roberts JM, Lain KY, Roberts JM, Lain KY. Recent Insights into the pathogenesis of preeclampsia. *Placenta*. 2002; 23:359–372. [PubMed: 12061851]
4. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *New England Journal of Medicine*. 2004; 350:672–683. [PubMed: 14764923]
5. Hellske S, Vuorela P, Carpen O, Hornig C, Weich H, Halmesmaki E, et al. Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. *Molecular Human Reproduction*. 2001; 7:205–210. [PubMed: 11160848]
6. Tsatsaris V, Goffin F, Munaut C, Brichant JF, Pignon MR, Noel A, et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. *Journal of Clinical Endocrinology & Metabolism*. 2003; 88:5555–5563. [PubMed: 14602804]
7. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *Journal of Clinical Investigation*. 2003; 111:649–658. [PubMed: 12618519]
8. Stepan H, Unversucht A, Wessel N, Faber R, Stepan H, Unversucht A, et al. Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion. *Hypertension*. 2007; 49:818–824. [PubMed: 17261644]
9. Staff AC, Braekke K, Harsem NK, Lyberg T, Holthe MR, Staff AC, et al. Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during pre-eclampsia. *European Journal of Obstetrics, Gynecology, & Reproductive Biology*. 2005; 122:33–39.
10. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee KY, Goncalves LF, et al. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. *Young Investigator Award. American Journal of Obstetrics & Gynecology*. 2004; 190:1541–1547. [PubMed: 15284729]
11. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *New England Journal of Medicine*. 2006; 355:992–1005. [PubMed: 16957146]
12. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *Journal of Maternal-Fetal & Neonatal Medicine*. 2008; 21:9–23. [PubMed: 18175241]
13. McKeeman GC, Ardill JE, Caldwell CM, Hunter AJ, McClure N, McKeeman GC, et al. Soluble vascular endothelial growth factor receptor-1 (sFlt-1) is increased throughout gestation in patients who have preeclampsia develop. *American Journal of Obstetrics & Gynecology*. 2004; 191:1240–1246. [PubMed: 15507947]
14. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM, et al. The Danish National Birth Cohort--its background, structure and aim. *Scandinavian Journal of Public Health*. 2001; 29:300–307. [PubMed: 11775787]
15. Klemmensen AK, Olsen SF, Osterdal ML, Tabor A, Klemmensen AK, Olsen SF, et al. Validity of preeclampsia-related diagnoses recorded in a national hospital registry and in a postpartum interview of the women. *American Journal of Epidemiology*. 2007; 166:117–124. [PubMed: 17556761]
16. van Eijsden M, van der Wal MF, Hornstra G, Bonsel GJ, van Eijsden M, van der Wal MF, et al. Can whole-blood samples be stored over 24 hours without compromising stability of C-reactive protein, retinol, ferritin, folic acid, and fatty acids in epidemiologic research? *Clin Chem*. 2005; 51:230–232. [PubMed: 15613719]
17. Wood, SN. *Generalized Additive Models: An Introduction with R*. Chapman and Hall. CRC Press; 2006.

18. Rana S, Karumanchi SA, Levine RJ, Venkatesha S, Rauh-Hain JA, Tamez H, et al. Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia.[see comment]. *Hypertension*. 2007; 50:137–142. [PubMed: 17515455]
19. Su YN, Lee CN, Cheng WF, Shau WY, Chow SN, Hsieh FJ, et al. Decreased maternal serum placenta growth factor in early second trimester and preeclampsia. *Obstetrics & Gynecology*. 2001; 97:898–904. [PubMed: 11384693]
20. Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E, et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. *Journal of Maternal-Fetal & Neonatal Medicine*. 2009; 22:1021–1038. [PubMed: 19900040]
21. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA, et al. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. *American Journal of Obstetrics & Gynecology*. 2003; 188:177–182. [PubMed: 12548214]
22. Tjoa ML, van Vugt JM, Mulders MA, Schutgens RB, Oudejans CB, van Wijk IJ, et al. Plasma placenta growth factor levels in midtrimester pregnancies.[see comment]. *Obstetrics & Gynecology*. 2001; 98:600–607. [PubMed: 11576575]
23. Tidwell SC, Ho HN, Chiu WH, Torry RJ, Torry DS, Tidwell SC, et al. Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. *American Journal of Obstetrics & Gynecology*. 2001; 184:1267–1272. [PubMed: 11349200]
24. Unal ER, Robinson CJ, Johnson DD, Chang EY. Second-trimester angiogenic factors as biomarkers for future-onset preeclampsia. *American Journal of Obstetrics and Gynecology*. 2007; 197:211. [PubMed: 17689655]
25. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, et al. First Trimester Placental Growth Factor and Soluble Fms-Like Tyrosine Kinase 1 and Risk for Preeclampsia. *J Clin Endocrinol Metab*. 2004; 89:770–775. [PubMed: 14764795]
26. Sattar N, Greer IA. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? *BMJ*. 2002; 325:157–160. [PubMed: 12130616]
27. Montes A, Walden CE, Knopp RH, Cheung M, Chapman MB, Albers JJ, et al. Physiologic and supraphysiologic increases in lipoprotein lipids and apoproteins in late pregnancy and postpartum. Possible markers for the diagnosis of "prelipemia". *Arteriosclerosis*. 1984; 4:407–417. [PubMed: 6431954]
28. Kaaja R, Laivuori H, Laakso M, Tikkanen MJ, Ylikorkala O, Kaaja R, et al. Evidence of a state of increased insulin resistance in preeclampsia. *Metabolism: Clinical & Experimental*. 1999; 48:892–896. [PubMed: 10421232]
29. Barden AE, Beilin LJ, Ritchie J, Walters BN, Michael C, Barden AE, et al. Does a predisposition to the metabolic syndrome sensitize women to develop pre-eclampsia? *Journal of Hypertension*. 1999; 17:1307–1315. [PubMed: 10489109]
30. Ness RB.; Powers, RW. *Epidemiology of hypertension*. Stanford: Appleton & Lange; 1999.
31. Eskenazi B, Fenster L, Sidney S. A multivariate analysis of risk factors for preeclampsia. *JAMA*. 1991; 266:237–241. [PubMed: 2056625]
32. Rey E, Couturier A, Rey E, Couturier A. The prognosis of pregnancy in women with chronic hypertension. *American Journal of Obstetrics & Gynecology*. 1994; 171:410–416. [PubMed: 8059820]
33. Ness RB, Markovic N, Bass D, Harger G, Roberts JM, Ness RB, et al. Family history of hypertension, heart disease, and stroke among women who develop hypertension in pregnancy. *Obstetrics & Gynecology*. 2003; 102:1366–1371. [PubMed: 14662228]
34. Haggerty CL, Ferrell RE, Hubel CA, Markovic N, Harger G, Ness RB. Association between allelic variants in cytokine genes and preeclampsia. *American Journal of Obstetrics & Gynecology*. 2005; 193:209–215. [PubMed: 16021081]

Demographic, clinical, and behavioral characteristics, and pregnancy outcomes of primiparous preeclamptic cases and normotensive controls

Table 1

	Preeclampsia (N=211)	Normotensive controls (N=123)	OR (95% CI) or p-value
Gestational age at serum collection (mean $\pm$ sd)	17.7 $\pm$ 3.2	17.2 $\pm$ 3.0	p=0.06
Maternal Age			
<= 25	n=52 24.6%	n=39 18.3%	1.0
26-30	n=113 53.6%	n=127 59.6%	0.7 (0.4 – 1.1)
31-35	n=40 19.0%	n=42 19.7%	0.7 (0.4 – 1.3)
36+	n=6 2.8%	n=5 2.3%	0.9 (0.3 – 3.2)
BMI			
< 25	n=129 61.1%	n=169 79.3%	1.0
>=25 and < 30	n=49 23.2%	n=37 17.4%	1.7 (1.1 – 2.8)
>=30	n=33 15.6%	n=7 3.3%	6.2 (2.6 – 14.4)
Smoking			
No	n=181 85.8%	n=167 78.4%	1.0
Yes, past	n=16 7.6%	n=17 8.0%	0.9 (0.4 – 1.8)
Yes, current	n=14 6.6%	n=29 13.6%	0.4 (0.2 – 0.9)
Gestational age at delivery (mean $\pm$ sd)	38.2 $\pm$ 2.3	39.6 $\pm$ 1.8	p<0.0001
Small for gestational age	n=17 8.1%	n=17 8.0%	p=0.98
Preterm Birth <37 weeks	n=45 21.3%	n=10 4.7%	p<0.0001
Preterm Birth < 34 weeks	n=8 3.8 %	n=4 1.9%	p=0.24

Table 2

Relationship between sFlt1, sEng and preeclampsia within the second trimester

<i>Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1256.0 (682.4)	211	1173.1 (592.2)	213	1068.2	0.10
sEng (ng/mL)	4.3 (1.1)	211	4.1 (1.1)	213	3.9	0.09
<i>Severe Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1332.7 (769.5)	59	1173.1 (592.2)	213	1068.2	0.04
sEng (ng/mL)	4.4 (1.2)	59	4.1 (1.1)	213	3.9	0.05
<i>Preterm (<37 weeks) Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1195.2 (772.9)	45	1173.1 (592.2)	213	1068.2	0.97
sEng (ng/mL)	4.4 (1.2)	45	4.1 (1.1)	213	3.9	0.16
<i>Early Preterm (<34 weeks) Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	915.6 (402.6)	8	1173.1 (592.2)	213	1068.2	0.22
sEng (ng/mL)	4.0 (0.8)	8	4.1 (1.1)	213	3.9	0.99
<i>Preeclampsia with SGA<5th percentile</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1311.2 (500.6)	17	1173.1 (592.2)	213	1068.2	0.21
sEng (ng/mL)	4.8 (0.8)	17	4.1 (1.1)	213	3.9	p<.0001

a_p based on log transformed sFlt1 and sEng, base 2

Table 3

Relationship between sFlt1, sEng and preeclampsia between 20 and 27 weeks gestation

<i>Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1421.2 (745.9)	60	1313.1	1168.1 (537.5)	47	1142.5
sEng (ng/mL)	4.3 (1.1)	60	4.1	4.2 (1.3)	47	3.9
						0.45
<i>Severe Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1357.6 (572.8)	18	1295.3	1168.1 (537.5)	47	1142.5
sEng (ng/mL)	4.3 (1.4)	18	4.0	4.2 (1.3)	47	3.9
						0.72
<i>Preterm (<37 weeks) Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1221.5 (679.2)	12	942.8	1168.1 (537.5)	47	1142.5
sEng (ng/mL)	4.6 (1.3)	12	4.2	4.2 (1.3)	47	3.9
						0.26
<i>Early Preterm (<34 weeks) Preeclampsia</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	913.0 (417.2)	2	913.0	1168.1 (537.5)	47	1142.5
sEng (ng/mL)	4.5 (0.4)	2	4.5	4.2 (1.3)	47	3.9
						0.53
<i>Preeclampsia with SGA<5th percentile</i>			<i>No PE</i>			<i>a_p-value</i>
Mean (SD)	N	Median	Mean (SD)	N	Median	
sFlt1 (pg/mL)	1414.5 (671.5)	6	1428.6	1168.1 (537.5)	47	1142.5
sEng (ng/mL)	4.4 (0.4)	6	4.3	4.2 (1.3)	47	3.9
						0.09

a_p based on log transformed sFlt1 and sEng, base 2