Angiogenic biomarkers for risk stratification in women with suspected preeclampsia
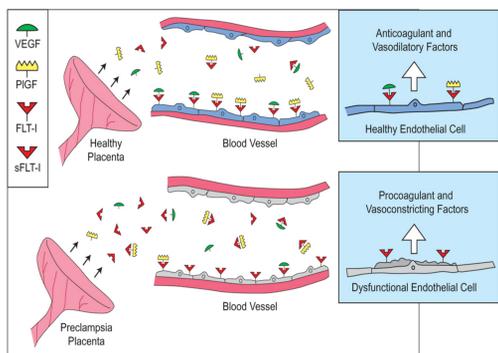
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Introduction

- Preeclampsia is a syndrome defined by the new onset of hypertension (SBP >140 mmHg or DBP >90 mmHg) and proteinuria (24 hour urine protein >300 mg) after 20 weeks of gestation.
- Affects 3-5% of all pregnancies can result in serious maternal and neonatal morbidity and mortality.
- Delivery of the neonate is the only definitive treatment, thus preeclampsia is a major cause of preterm birth.
- Although gestational hypertension is common, only a minority eventually develop severe preeclampsia with serious complications.
- A blood test which could predict maternal or neonatal complications in women with suspected preeclampsia would be useful for clinical risk stratification.
- **Pathophysiology of preeclampsia**:

Materials and Methods

- A **single-center prospective cohort study of 315 pregnant women** who presented to GWUH Labor and Delivery service with a clinical suspicion of preeclampsia between February 2007 and November 2011. Informed consent was obtained.

- Baseline demographic information and medical history was collected on each patient including maternal age, ethnicity, body mass index, tobacco and other substance use, gestational age, medical problems and obstetric history.

- Serum samples were obtained from each enrolled subject within 24 hours of admission, and sFlt1, PlGF and sEng ELISA assays were performed in duplicate by a blinded laboratory technician at the University of Massachusetts.

- **Primary outcome variable**: Preeclampsia defined as the new onset of hypertension and proteinuria after 20 weeks gestation, according to guidelines published by NHLBI and ACOG. Hypertension was defined as SBP≥140 or DBP≥90 on at least 2 occasions at least 4 hours apart. Proteinuria was defined as >300mg of protein in a 24hr urine specimen, spot urine protein:creatinine ratio >0.3, or urine dipstick of 1+ or higher. Additionally:
  - Women with chronic hypertension and without proteinuria prior to 20 wks gestation: diagnosis of preeclampsia required new onset proteinuria after 20 wks gestation
  - Women with baseline proteinuria, but without chronic hypertension: diagnosis of preeclampsia required new onset hypertension and worsening proteinuria
  - Women with chronic hypertension and baseline proteinuria prior to 20 wks: preeclampsia required worsening hypertension and one of the following: (1) elevated AST or ALT; (2) worsening proteinuria; (3) thrombocytopenia; or (4) eclampsia

- **Secondary outcome** was a composite of adverse maternal and neonatal complications, including any one of the following. Maternal complciations: elevated liver enzymes, thrombocytopenia, oliguria, seizures, pulmonary edema or cerebral hemorrhage. Neonatal complications: early preterm birth (<34 weeks), placental abruption, small for gestational age, NICU admission, fetal or neonatal demise.

- **Statistical analysis**: Wilcoxon rank sum tests were used to compare serum levels of biomarkers among women with and without pre-eclampsia. A Kruskal-Wallis test with post-hoc comparisons was used to do a three-way comparison of the serum levels of the angiogenic factors between women without preeclampsia, women with preeclampsia and no complications and women with preeclampsia and complications. A logistic regression analysis, adjusted for race, obesity, renal disease, chronic hypertension, gestational age at time of sample collection was used to identify correlating variables.

Results

Table 1. Characteristics of women who were enrolled in the study and later identified to have or not have preeclampsia.

Variable	Pre-eclampsia (n=85)	No pre-eclampsia (n=217)
Mean maternal age in years (SE)	29.5 (0.7)	30.8 (0.4)
Race	% (N)	% (N)
White	18 (15)	27 (59)
Black	71 (60)	69 (133)
Other	12 (10)	12 (26)
Smoking		
Never	82 (70)	92 (201)
Former	13 (11)	5 (11)
Current	5 (4)	3 (6)
Chronic HTN	35 (30)	20 (43)
Diabetes	6 (5)	6 (13)
Renal disease	2 (2)	2 (4)
Gravidity (excluding current pregnancy)		
0	33 (28)	32 (69)
1-3	44 (37)	51 (111)
4+	24 (20)	17 (37)
Prior history of preeclampsia	15 (13)	10 (21)
Delivery method		
C-section	58 (49)	49 (111)
Vaginal induced	24 (20)	25 (54)
Vaginal spontaneous	19 (16)	26 (57)
Gestational age at delivery (weeks)		
37+	62 (53)	76 (163)
<37	38 (32)	24 (51)

*17 women were excluded due to failure to contribute a blood sample

Table 4 Legend. Logistic regression analysis was performed and adjusted for race, renal disease, hypertension status, gravity and prior history of pre-eclampsia. Significant correlations are indicated in bold.

Table 2. Serum levels of PlGF are significantly lower in women with preeclampsia compared to women without pre-eclampsia.

Variable	No Pre-eclampsia (n=215)	Pre-eclampsia (n=83)	Wilcoxon p-value
PlGF	**155 (3-3575)**	**117 (8.5-797)**	**0.0295**
sFlt1/PlGF	69.4 (2.2-1619)	95.7 (2-1982)	0.0843
sFlt1	9609 (140-119,564)	10689 (939-76,154)	0.56
Eng	15.5 (2.5, 297)	20.3 (3.2-161)	0.17

Serum samples were processed as per methods and analyzed by ELISA. Statistical analysis showed that PlGF levels are significantly lower in the pre-eclamptic women. Serum levels of Sflt1 and sEng are not significantly different between the two groups. The ratio of Sflt1 to PlGF did not reach significance when compared between groups, though a trend towards an increase was noted (p=0.08).

Results, cont.

Table 3 Serum levels of PlGF are significantly lower in women with pre-eclampsia and even lower in women with pre-eclampsia and complications compared with women with gestational hypertension alone.

Variable	No Preeclampsia (n=215)	Preeclampsia only (n=60)	Preeclampsia + complications (n=24)	Kruskal-Wallis p-value
PlGF	**155 (3-3575)**	**129 (24-798)**	**78 (8.5-653)**	**0.0285**
sFlt/PlGF	69.4 (2.2-1619)	79.5 (3.3-470)	145 (2-1985)	0.11
sFlt1	9609 (140-119,564)	10,674 (1904-26,774)	10,123 (959-76,154)	0.87
sEng	15.5 (2.5, 297)	18.6 (3.6-65)	32.4 (3.2-161)	0.28

Serum levels of PlGF are significantly lower in women with preeclampsia compared to women without. Most importantly, PlGF is further decreased in women with both preeclampsia and documented maternal and neonatal complications. Serum levels of Sflt1 and Eng were not significantly different among the two groups and there was no difference in serum levels among the women who developed complications. The ratio of Sflt to PlGF failed to show a statistically significant difference between any groups.

Table 4. Serum levels of PlGF, gestational age, current smoking status and maternal age are correlated with an increased incidence of preeclampsia.

Variable	Adjusted OR	95% CI
PlGF per 100 unit change	**0.83**	**0.69, 0.99**
Race		
Black vs. White	1.04	0.48, 2.26
Other vs. White	1.22	0.44, 3.37
Gestational age at sample (wks)	**0.91**	**0.85, 0.98**
Gravity	1.07	0.93, 1.25
Preeclampsia history	1.12	0.46, 2.69
History of renal disease	0.35	0.05, 2.56
Chronic hypertension	1.62	0.84, 3.11
Smoking status		
Former vs. Never	1.85	0.46, 7.37
Current vs. Never	**4.43**	**1.64, 11.95**
Maternal age (yrs)	**0.95**	**0.90, 0.99**

Discussion

- Among a cohort of women presenting acutely with signs and symptoms suggestive of preeclampsia, women who eventually met diagnostic criteria for preeclampsia had lower serum PlGF levels at presentation as compared to women who did not meet preeclampsia criteria.

- sFlt1, sEng, and the sFlt1/PlGF ratio were not significantly different in women who did vs. those who did not meet criteria for preeclampsia

- Women who developed preeclampsia with maternal or neonatal complications had lower serum PlGF levels at presentation as compared to women who developed preeclampsia without complications and women who did not meet criteria for preeclampsia.

- Serum PlGF may be a useful tool for risk stratification in women who present with signs/symptoms suggestive of preeclampsia.

- This tool, rooted in the fundamental pathogenesis of preeclampsia, could arm clinicians with a significant advance in the identification of high-risk patients, ensuring that women with a high likelihood of developing severe preeclampsia complications receive aggressive monitoring and intervention, while allowing women at low risk of complications to be managed conservatively.

- This is the first study to evaluate the utility of biomarkers for risk stratification in the triage setting. Larger studies are needed to confirm our result, and to establish the utility of PlGF for risk stratification in the clinical setting.

References

1. Sibai B. M., *et al.* N Engl J Med 1998;339:667-71.
2. Maynard, S.E., *et al.* J Clin Invest 2003;111:649-58.
3. Levine, R.J., *et al.* N Engl J Med 2004;350:672-83.
4. Levine, R.J., *et al.* N Engl J Med 2006;355:992-1005.
5. Maynard SE, et al. J Clin Invest 111(5): 649-658, 2003)
6. Maynard SE and Karumanchi SA. Seminars in Nephrology, 2011; 31:33-46.
7. Moore Simas TA, *et al.* Am J Obstet Gynecol 2007;197(3): 244 e241-248,
8. Kim SY, *et al.* J Korean Med Sci 2007; 22(5): 873-877.

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