

ORIGINAL ARTICLE

The association of circulating angiogenic factors and HbA1c with the risk of preeclampsia in women with preexisting diabetes

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Objective: To assess whether glycemic control, soluble fms-like tyrosine kinase 1 (sFlt1) and placental growth factor (PlGF) were associated with the development of preeclampsia (PE) or gestational hypertension (GHTN) in women with preexisting diabetes. *Methods:* Maternal circulating angiogenic factors (sFlt1 and PlGF) measured on automated platform were studied at four time points during pregnancy in women with diabetes ($N=159$) and reported as multiples of the median (MOM) of sFlt1/PlGF ratio (median, 25th–75th percentile) noted in non-diabetic non-hypertensive control pregnant population ($N=139$). Diagnosis of PE or GHTN was determined by review of de-identified clinical data. *Results:* PE developed in 12% ($N=19$) and GHTN developed in 23% ($N=37$) of the women with diabetes. Among diabetic women without PE or GHTN, median sFlt1/PlGF levels at 35–40 weeks was threefold higher than in non-diabetic controls [MOM 3.21(1.19–7.24), $p=0.0001$]. Diabetic women who subsequently developed PE had even greater alterations in sFlt1/PlGF ratio during the third trimester [MOM for PE at 27–34 weeks 15.18 (2.37–26.86), at 35–40 weeks 8.61(1.20–18.27), $p \leq 0.01$ for both windows compared to non-diabetic controls]. Women with diabetes who subsequently developed GHTN also had significant alterations in angiogenic factors during third trimester; however, these findings were less striking. Among women with diabetes, glycosylated hemoglobin (HbA1c) during the first trimester was higher in subjects who subsequently developed PE (7.7 vs 6.7%, $p=0.0001$ for diabetic PE vs diabetic non-PE). *Conclusions:* Women with diabetes had a markedly altered anti-angiogenic state late in pregnancy that was further exacerbated in subjects who developed PE. Altered angiogenic factors may be one mechanism for the increased

risk of PE in this population. Increased HbA1c in the first trimester of pregnancies in women with diabetes was strongly associated with subsequent PE.

Keywords Angiogenesis, Diabetes, HbA1C, PlGF, Preeclampsia, sFlt1.

INTRODUCTION

Hypertensive disorders of pregnancy are one of the most common direct causes of maternal and neonatal morbidity and mortality worldwide (1). Complications include placental abruption, hemolysis, elevated liver enzymes and low platelets syndrome, acute renal failure, eclampsia, preterm delivery, fetal growth restriction, perinatal and maternal death.

Preeclampsia (PE) is a syndrome of new onset hypertension (HTN) and proteinuria after 20 weeks of gestation in a previously normotensive female without proteinuria. PE affects about 5% of pregnancies worldwide, and the risk is about 3–4 fold more common in women with preexisting diabetes mellitus (DM) (2). The risk of PE increases with increasing severity of preexisting DM, as graded by the White classification (2–5). It is known that microalbuminuria (6,7), nephropathy (8), retinopathy (4,5,9,10) and HTN (11,12) increase the risk for PE in women with preexisting DM.

There is relatively limited data on the association of glycemic control in pregnancy and PE. A recent study (13) of 749 women with type 1DM showed that women who developed PE had higher glycosylated hemoglobin (HbA1c) values before pregnancy and both early and late in pregnancy. Prior smaller studies had shown that glycemic control, early (5,10) and late (14,15), in pregnancy impacts risk of PE. There is a paucity of data related to glycemic control and the risk of PE in women with type 2DM.

Although the etiology of PE is not fully understood, recent studies have shown that PE is associated with altered levels of angiogenic factors, including increased levels of soluble fms-like tyrosine kinase 1 (sFlt1) and reduced levels of placental growth factor (PlGF) (16). PlGF is a placenta-derived angiogenic factor, and sFlt1 is an alternatively spliced circulating form of the VEGF receptor that binds and reduces bioactivity of PlGF. Abnormalities in these circulating factors that regulate angiogenesis have been reported in PE (16). It is not clear if this is true for women with DM who develop PE. These changes have been shown to occur up to 5 weeks prior to the onset of clinical PE in patients who are low risk for PE and are thought to be useful in predicting preterm PE. There is limited data on angiogenic factors in high risk patients, including women with preexisting DM. In a large study that included women at high risk of PE, there were modest differences in sFlt-1 and PlGF between those who develop PE versus those who do not (17). In another study of 151 women with type 1DM (18), elevations in sFlt-1 and reductions in PlGF were found prior to the onset of PE, in a similar fashion to those previously described in low risk patients (16). However, it is not known whether the levels of angiogenic factors are altered by DM *per se* and if this could explain the increased risk for PE in this pregnant population.

The aim of this study was to assess levels of angiogenic factors (sFlt1 and PlGF) in women with preexisting DM compared to women without DM.

We also assessed the risk of PE and GHTN in relation to angiogenic factors and HbA1c in women with preexisting DM.

RESEARCH DESIGN AND METHODS

Subjects

Type 1 or 2 diabetic pregnant women were recruited from the Joslin and Beth Israel Deaconess Medical Center (BIDMC) Diabetes in Pregnancy Program in Boston, MA, during the period of August 2004 and May 2008. Non-diabetic pregnant women presenting to BIDMC contemporaneously were recruited as controls. All subjects provided their informed consent prior to participation in the study. The study was approved by the Institutional Review Board at BIDMC.

Women with preexisting type 1 or 2 diabetes (DM group) ($n=159$) with singleton pregnancies were recruited during first trimester of pregnancy and studied. Of these women, 117 (74%) had type 1 DM and 42 (26%) had type 2 DM. Blood samples were collected from subjects at routine clinic visits throughout their pregnancy at time intervals when they would be having standard blood testing. Samples were collected at their first prenatal visits (7–14 weeks), at 16–20 weeks when triple screen was performed, at 24–32 weeks when complete blood count was drawn with an oral glucose tolerance test in normal subjects and immediately prior to delivery. Plasma or serum samples were frozen at -80°C and thawed once for analysis of angiogenic markers. Contemporaneous control subjects, without preexisting DM and who did not develop any hypertensive complications ($N=139$) were also studied to generate reference levels of angiogenic factors throughout gestation.

De-identified medical records were extracted and reviewed by study physicians. A team of three maternal–fetal medicine specialists determined if subjects had PE (de novo or superimposed) or GHTN. PE was defined as follows: systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg diastolic after 20 weeks gestation and proteinuria > 300 mg/24 h or $> 2+$ on urine dip-stick or protein/creatinine ratio > 0.3 in a woman with previously normal blood pressure. GHTN was defined as SBP ≥ 140 or DBP ≥ 90 after 20 weeks gestation and no proteinuria (19). In subjects who were normotensive but had proteinuria at baseline, the diagnosis of PE required the presence of thrombocytopenia, an aspartate aminotransferase (AST) > 70 units/l or HTN accompanied by headaches, epigastric pain or sudden increase in proteinuria (five times baseline value or twice baseline if it was > 5 g per 24 h). In women with both HTN and proteinuria at baseline, the diagnosis of PE required any of the following: thrombocytopenia, and elevated AST > 70 units/l, or worsening HTN (as shown by two DBPs ≥ 100 mm Hg taken 4 h apart in the week before delivery) combined with either exacerbation of proteinuria (as above), severe headaches or epigastric pain (20).

History of retinopathy was defined as any degree of retinopathy documented in the medical record, either by direct review of dilated eye exam report or documentation of a history of retinopathy in the patient medical record, prior to the onset of pregnancy. Severity of retinopathy was not assessed. History of HTN was defined as HTN documented in the medical record prior to the

onset of pregnancy. Finally, nephropathy was defined as a history of either spot albumin/creatinine ratio >300 mcg/mg or a protein/creatinine >0.3 , documented in the medical record, either by laboratory value or documentation by a physician in the medical record, prior to onset of pregnancy or at the initial pregnancy visit.

HbA1c Measurements

HbA1c was measured during office visits beginning during first trimester as part of routine clinical care throughout pregnancy for women with DM. Assays for the HbA1c were measured at the using standard commercial assays (Roche Hitachi Tina-quant immunoassay (Basel, Switzerland or the Roche Integra Hemoglobin A1C Generation 2 immunoassay (Basel, Switzerland).

Angiogenic Factors Assays

Specimens were frozen at -80°C and thawed once for performance of the assays. Ethylenediaminetetraacetic acid plasma or serum specimens were analyzed for free sFlt1 and free PlGF using automated prototype assays of Access Immunoassay systems (Beckman Coulter, Chaska, MN). All samples were collected during the 4 years of the study, including the contemporaneous controls. The access PlGF and sFlt1 assays are one-step immunoenzymatic “sandwich” assays and have been described previously (21). The upper limit of the measuring range for the prototype PlGF assay was 10 000 pg/mL, while the upper limit of the measuring range for the prototype sFlt1 assay was 132 500 pg/mL. Within run and between run precision has been determined previously with 3% CV for each assay (22). All assays were performed in one batch by a technician who was unaware of the clinical outcomes. The results are reported as sFlt1/PlGF ratio, which is an index of anti-angiogenic activity related to sFlt1 and its ligands. The ratio takes into account both the increase in sFlt1 and the decrease in PlGF in women who develop PE and appears to be a more reliable index for the prediction of PE than either protein alone (16,23).

Statistical Analysis

Since this was an exploratory study, there were no data available for the angiogenic factors in diabetic pregnant population, we did not perform a power analyses. Characteristics of diabetic women with and without PE or GHTN were summarized using means (standard deviations) or percents (sample size), where appropriate, and subsequently compared using independent samples *t*-tests or chi-squared tests. All continuous variables were examined for normality. Gestational weight for gestational age was calculated according to methods reported elsewhere (24). *p* Values presented are for each group compared to non diabetic subjects and compared to diabetic subjects without pre-eclampsia. sFlt1/PlGF ratios among the three subgroups are expressed as multiples of the median (MOM) of non-diabetic control women and analyzed cross-sectionally at four gestational windows 11–18 weeks, 19–26 weeks, 27–34 weeks and 35–40 weeks. The reference values in all MOM calculations were derived from angiogenic markers of healthy non-diabetic control women who presented contemporaneously to the obstetric clinic. Mann–Whitney *U* tests were utilized for all MOM comparisons. Univariate and multivariable

(adjusted for parity and body mass index) log-binomial regression models were used to predict the relative risk of pre-eclampsia by various clinically relevant cut-offs for HbA1c. *p* Values <0.05 were considered statistically significant. All analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Middleton, MA).

RESULTS

The clinical characteristics of all subjects with DM are shown in Table 1. In this study, 19 (12%) subjects with DM developed PE and 37 (23%) subjects with diabetes developed GHTN. There were no differences in preconception/first prenatal visit BMI, race, smoking history or years of diabetes in subjects with DM–non PE, DM–PE or DM–GHTN. In the DM–PE subjects, 36.8% had a history of HTN, whereas only 15.5% of DM–non PE ($p=0.02$) had a history of HTN. First trimester retinopathy was present in 42.1% DM–PE versus 26.2% DM–non PE ($p=0.007$), and third trimester retinopathy was present in 47.4% DM–PE versus 30.1% DM–non PE ($p=0.002$). Third trimester retinopathy was present in 46% of subjects with DM–GHTN versus 30.1% of those with DM–non PE and no GHTN ($p=0.04$). Prior history of PE was present in 26.3% of subjects with DM–PE and 10.7% of those DM–non PE ($p=0.02$). Nephropathy was present in 21.1% of those with DM–PE and 12.6% of those with DM–non PE, which was not significant ($p=0.15$). Women with DM–PE compared with diabetic non-PE also had increased rates of having an infant with lower birth weight, lower birth weight for gestational age and earlier delivery. Clinical characteristics of the non-diabetic control population are also listed in Table 1.

Circulating Angiogenic Factors

Angiogenic factors in the three groups of subjects – DM–non PE, DM–PE and DM–GHTN are shown as MOM values of the analyte concentration in control pregnant women without DM at each gestational window (Table 2). DM–non-PE subjects, compared to women without DM had slightly higher sFlt1/PlGF ratios levels at 11–18 weeks, however, had three fold higher sFlt1/PlGF ratios at 35–40 weeks. The DM-PE group had 15-fold higher sFlt1/PlGF ratios during 27–34 weeks and ninefold higher sFlt1/PlGF ratios at 35–40 weeks compared with women without diabetes. While these differences were also noted in DM–GHTN group, the magnitude was less striking. Interestingly, the relatively higher sFlt1/PlGF ratios in the DM–PE group as compared to DM–GHTN group was largely driven by differences in PlGF rather than altered sFlt1. There were no significant differences in angiogenic factors early in pregnancy in the DM–PE group or in the DM–GHTN group.

HbA1c and PE

HbA1c at the first prenatal visit (baseline) was higher in the DM–PE group versus DM–non PE (7.7 vs. 6.7; $p=0.0001$), while there was no difference in HbA1c at delivery (6.2 in PE vs. 6.1 No PE). There was no difference in HbA1c levels in DM subjects with GHTN at baseline compared to DM without PE or GHTN. Relative risk for PE in DM subjects was assessed based on baseline HbA1c values both in a univariate and multivariable analysis (Table 3).

Table 1. Characteristics of diabetic subjects with and without hypertensive complications and non-diabetic controls.

	Non-diabetic controls (n = 139)	Diabetes non-PE (n = 103)	Diabetes PE (n = 19)	Diabetes GHTN (n = 37)	p Value*	p Value*
Mother's age (years)	33.7 ± 4.7	32.6 ± 5.2	31.3 ± 5.7	32.4 ± 5.9	0.33	0.86
BMI (Prior to or at first PNV)	24.5 ± 4.2	28.2 ± 6.0	31.8 ± 10.1	27.1 ± 3.8	0.15	0.23
Race (%)					0.32	0.29
Caucasian	80.6 (112)	86.4 (89)	73.7 (14)	97.3 (36)		
Black	5.0 (7)	4.9 (5)	15.8 (3)	0.0 (0)		
Asian	10.8 (15)	2.9 (3)	5.3 (1)	0.0 (0)		
Hispanic	1.4 (2)	5.8 (6)	5.3 (1)	2.7 (1)		
Smoker (%)	1.4 (2)	7.8 (8)	15.8 (3)	2.7 (1)	0.26	0.28
History of hypertension (%)	1.4 (2)	15.5 (16)	36.8 (7)	2.7 (1)	0.02†	0.04†
Refinopathy – first (%)		26.2 (27)	42.1 (8)	40.5 (15)	0.007†	0.09
Refinopathy – third (%)		30.1 (31)	47.4 (9)	46.0 (17)	0.002†	0.04†
Nephropathy (%)		12.6 (13)	21.1 (4)	0.0 (0)	0.15	0.02†
History of preeclampsia (%)	5.8 (8)	10.7 (11)	26.3 (5)	5.4 (2)	0.02†	0.34
Parity	1 ± 1	1 ± 1	1 ± 1	1 ± 1	0.57	0.64
Years with DM		12.9 ± 8.9	15.4 ± 9.3	15.0 ± 7.7	0.29	0.19
HbA1C (baseline)		6.7 ± 1.1	7.7 ± 1.1	7.0 ± 1.6	0.001†	0.32
HbA1C (delivery)		6.1 ± 0.6	6.2 ± 0.4	6.4 ± 0.8	0.41	0.03†
Type 1 DM (%)		72.8 (75)	68.4 (13)	78.4 (29)	0.65	0.56
Gestational age in weeks at delivery	38.9 ± 1.5	37.2 ± 2.2	35.9 ± 2.0	37.5 ± 1.3	0.01†	0.44
C-section (%)	38.9 (54)	76.7 (79)	94.7 (18)	86.5 (32)	0.08	0.24
Baby weight (g)	3346 ± 455	3601 ± 779	2958 ± 669	3652 ± 576	0.001†	0.68
BW for GA (percentile)	48.9 ± 24.9	72.4 ± 26.1	54.0 ± 28.7	76.4 ± 23.9	0.007†	0.43
Baby gender (males) (%)	49.6 (69)	41.8 (43)	31.6 (6)	51.4 (19)	0.48	0.27
SBP at first prenatal visit	113 ± 10	115 ± 11	125 ± 13	119 ± 8	0.001†	0.02†
DBP at first prenatal visit	70 ± 8	71 ± 8	72 ± 8	74 ± 6	0.43	0.005†

*Significance compared to diabetic non-PE.

†Significant at <0.05 level.

Table shows mean ± standard deviation or percent (sample size) where appropriate.

PE = preeclampsia, GHTN = gestational hypertension, BMI = body mass index, PNV = prenatal visit, DM = diabetes mellitus, BW = birth weight, GA = gestational age, SBP = systolic blood pressure, DBP = diastolic blood pressure.

Table 2. Multiple of the median (MOM)* for various angiogenic markers in diabetic pregnancies.

Markers	Gestational age (years)	Non-diabetic controls† (median)		Diabetes non-PE (MOM)		Diabetes PE (MOM)		Diabetes GHTN (MOM) N = 37		
		N = 139	N = 103	N = 103	N = 19	N = 19	N = 37	N = 37		
sFlt-1/PlGF	11-18	39.3	1.34 (0.66, 2.26)	1.49 (0.99, 3.05)	0.04¶	0.08	0.40	1.45 (0.57, 2.24)	0.10	0.82
	19-26	5.0	1.66 (0.83, 3.34)	1.10 (0.58, 2.00)	0.05	0.77	0.33	2.20 (0.75, 4.40)	0.08	0.58
	27-34	2.8	1.45 (0.78, 1.95)	1.518 (2.37, 26.86)	0.12	0.001¶	0.03¶	5.56 (0.94, 25.29)	0.01¶	0.14
sFlt-1	35-40	39.6	3.21 (1.19, 7.24)	8.61 (1.20, 18.27)	0.0001¶	0.01¶	0.32	6.17 (2.28, 10.87)	<0.0001¶	0.16
	11-18	2218	1.16 (0.58, 1.81)	0.94 (0.37, 1.17)	0.32	0.49	0.13	0.87 (0.57, 1.78)	0.96	0.45
	19-26	2080	1.28 (0.68, 2.18)	0.83 (0.31, 1.42)	0.25	0.22	0.05	1.27 (0.56, 2.17)	0.40	0.90
PlGF	27-34	1746	1.86 (0.89, 4.48)	2.78 (1.09, 5.93)	0.03¶	0.05	0.50	2.01 (0.80, 4.51)	0.11	0.98
	35-40	8266	2.21 (0.87, 3.76)	2.74 (0.75, 6.56)	0.001¶	0.12	0.86	2.89 (1.09, 4.69)	0.002¶	0.29
	11-18	78.7	0.80 (0.46, 1.30)	0.52 (0.38, 0.95)	0.39	0.09	0.17	0.60 (0.32, 1.26)	0.11	0.21
PlGF	19-26	412.3	0.84 (0.52, 1.31)	0.62 (0.33, 1.34)	0.18	0.15	0.32	0.68 (0.46, 1.19)	0.09	0.46
	27-34	581.6	1.01 (0.43, 1.68)	0.24 (0.20, 0.37)	0.75	<0.0001¶	0.001¶	0.75 (0.31, 1.41)	0.15	0.23
	35-40	181.3	0.66 (0.48, 1.39)	0.50 (0.32, 0.69)	0.004¶	0.001¶	0.04¶	0.59 (0.41, 0.83)	<0.0001¶	0.10

PE = preeclampsia, GHTN = gestational hypertension.

Tables shows median (Q1, Q3).

*Multiple of median of circulating concentrations in non-diabetic controls.

†Median concentrations of sFlt1 and PlGF in pg/ml and sFlt1/PlGF ratio.

‡Compared to non-diabetic controls.

§Compared to cases (diabetics) without PE and without GHTN.

¶Significant at $p < 0.05$.

Table 3. Relative risk of preeclampsia with HbA1C cut-offs among diabetics.

HbA1C	% (n) <HbA1C	% (n) ≥HbA1C	Univariate		Multivariable†	
			RR (95% CI)	p Value	RR (95% CI)	p Value
≥6.5	48.1 (76)	51.9 (82)	4.63 (1.40–15.38)	0.01*	4.42 (1.35–14.47)	0.01*
≥7	67.1 (106)	32.9 (52)	5.30 (2.00–14.07)	0.001*	5.26 (2.06–13.41)	0.001*
≥7.5	78.5 (124)	21.5 (34)	5.73 (2.41–13.66)	<0.0001*	5.60 (2.50–12.56)	<0.0001*
≥8	85.4 (135)	14.6 (23)	4.70 (2.08–10.64)	0.0002*	4.60 (2.18–9.69)	<0.0001*

*Significant at <0.05 level.

†Controlling for parity and body mass index.

Multivariable analysis controlled for parity and BMI. Subjects with high baseline HbA1c levels (as defined by various clinical cut-offs) have an increased risk for PE in both univariate and multivariable analyses compared to those with low HbA1c levels. The relative risk for PE in subjects with HbA1c levels ≥6.5% was 4.63 ($p=0.01$) in univariate analysis and 4.42 ($p=0.01$) in multivariable analysis. The risk of PE becomes more pronounced as HbA1c increased up until HbA1c > 8%, which only included 14% of subjects with DM in this study.

DISCUSSION

In this single center study, we found a prevalence rate of 12% PE and 23% GHTN, in line with rates reported in women with type 1 diabetes and type 2DM (3,5,11,12,14,25,26). Interestingly, circulating angiogenic factors during the third trimester were altered (threefold) in the diabetic women without hypertensive complications compared to the healthy women without diabetes. Women with DM who developed PE had even greater changes in angiogenic factors during the third trimester compared to healthy women without diabetes. Taken together with animal studies and epidemiological studies that have demonstrated altered angiogenic factors are related to the development of PE (27), we posit that the altered anti-angiogenic state in diabetic pregnancies may be one mechanism for the increased risk of PE in this population. However, changes in angiogenic factors were not noted early in pregnancy suggesting that these biomarkers are unlikely to be useful as a predictive test for PE in the DM population. This is in line with other larger prospective studies in the non-diabetic population that demonstrated relatively modest prediction of PE during early pregnancy (28–30).

Diabetic patients who developed PE did not have significant alterations in angiogenic factors early in pregnancy; however, sFlt1/PlGF ratio was dramatically elevated in the third trimester between 27 and 34 weeks. There is a large body of data in preeclamptic subjects without diabetes, which has shown that elevations in the sFlt1/PlGF ratio occur prior to the onset of clinical signs and symptoms (16,31). In a study of 151 subjects with type 1 DM, of which 20% developed PE, sFlt1 and PlGF levels were measured in each trimester and at term (18). There was an elevation in sFlt1 and the sFlt1/PlGF ratio, and a reduction in PlGF in the third trimester in those who developed PE

compared to those with type 1 DM who did not develop PE, consistent with our findings.

In addition, we also found that women with DM who develop PE have higher HbA1c levels at their first prenatal visit compared to those without PE, and that the HbA1c at time of delivery was not associated with PE. There is increased relative risk for PE when the HbA1c > 6.5% at baseline. This shows clearly that glucose control in early pregnancy is associated with the risk of PE, and that the risk increases as baseline HbA1c increases up to 8%, which was highest level that we could assess in our study due to the paucity of subjects with A1c > 8. Our data are also consistent with previously published data showing an increased risk of PE based on glycemic control early in pregnancy in women with type 1 diabetes (5,10,13), though the gestational ages varied slightly in different studies. A few studies showed that later glycemic control imparted risk for PE, rather than early gestation. Temple et al. found in women with type 1 DM that HbA1c at 12 weeks and 24 weeks increased risk, but first prenatal visit HbA1c did not (14), and Hsu et al. found that in women with type 1 DM, HbA1c at 16–20 weeks increased risk for PE but not earlier or later in gestation (15). There are limited data in women with preexisting type 2 DM. Cundy et al. found that in a group of women with type 1 and 2 DM, HbA1c > 9% at presentation was associated with an increased risk of PE (12). Sibai studied a cohort of type 1 and 2 diabetic subjects and showed increased risk of PE based on White classification, but did not assess glycemic control during pregnancy (32). Our study, which included 75% of subjects with type 1 DM and 25% with type 2 DM, demonstrated that there is a significant increase in risk for PE when HbA1c levels are $\geq 6.5\%$ at the first prenatal visit.

Our data also showed that women with DM who developed PE had higher rates of retinopathy both in the first and third trimesters, compared to those who did not develop PE. Retinopathy in our study included subjects with any degree of retinopathy, not just limited to proliferative retinopathy, as defined by White Class R. There is little published data on the risk of PE in women with diabetic retinopathy. Sibai showed that women with class R and/or F DM had increased rates of PE, but did not present data on risk with retinopathy alone (11). In a study by Howarth et al., 36% of subjects with type 1 DM had any degree of retinopathy, and retinopathy was significantly associated with the development of PE (4). There are also data showing worsening of retinopathy in subjects with type 1 diabetes who develop PE than those who do not develop PE (33). Our data demonstrating the increased risk of PE in subjects with retinopathy both in the first and third trimesters provides more information to use when counseling patients on their risk of PE.

Our study has some limitations. Data points in our study were primarily cross-sectional as not all subjects contributed to blood specimens at the various gestational windows. Because of sample size limitation, we were unable to study the various subgroups of PE in which sFlt1 and PlGF concentrations might have been altered even more. We were also unable to evaluate whether altered angiogenic factors are related to adverse maternal and perinatal adverse outcomes, as the majority of the patients developed PE close to term and did not develop adverse outcomes other than iatrogenic prematurity. In our study, only three preeclamptic subjects delivered at <32 weeks.

Therefore, we were unable to study the prediction of angiogenic markers for early PE similar to what has been reported in the non-diabetic population (28). Because of limitations in sample size, we were unable to specifically study the various subtypes of diabetes. Finally, we used either serum or plasma aliquots for the analyses of angiogenic factors that are sub-optimal as serum concentrations tend to have slightly higher values than plasma concentrations of angiogenic factors (34). Our study also does not shed light on the mechanisms of the altered angiogenic factors in diabetic pregnancies; however, we speculate that hyperglycemia may directly affect placental production of anti-angiogenic factors.

In summary, women with preexisting DM had higher sFlt1/PlGF ratios in the third trimester, potentially providing one mechanism for the increased risk of hypertensive complication in this population. Furthermore, subjects with preexisting type 1 or 2 DM with higher HbA1c early in pregnancy have a higher risk for developing PE. This may be clinically helpful in counseling these women about their risks for development of PE later in pregnancy. Further studies with larger samples sizes are needed to study specific subgroups of PE such as preterm PE and/or PE with growth restriction. Additional studies should also look at the utility of angiogenic markers in women with DM in which the clinical diagnosis of PE is unclear such as those with underlying HTN or proteinuria.

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DECLARATION OF INTEREST

Dr. Thadhani is a co-inventor on patents related to the prediction of preeclampsia that has been out licensed to diagnostic companies and has financial interest in Aggamin LLC. Dr. Karumanchi is a co-inventor of multiple patents related to angiogenic proteins for the diagnosis and therapy of preeclampsia. These patents have been licensed to multiple companies. Dr. Karumanchi reports having served as a consultant to Roche, Beckman Coulter, Siemens and has financial interest in Aggamin LLC. The remaining authors report no conflicts.

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