BMJ Open

Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032237
Article Type:	Research
Date Submitted by the Author:	09-Jun-2019
Complete List of Authors:	Zhu, Jing; MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China Zhang, Jun; Ministry of Education–Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine Ng, Mor Jack; KK Women's and Children's Hospital, Division of Obstetrics and Gynaecology Chern, Bernard; KK Women's and Children's Hospital Yeo, George S. H.; KK Women's and Children's Hospital, Singapore Tan, Kok Hian; KK Women's and Children's Hospital, Division of Obstetrics and Gyneacology
Keywords:	blood pressure, soluble fms-like tyrosine kinase 1, placental growth factor, preeclampsia

Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study

Jing Zhu,[1] Jun Zhang,[1,2] Mor Jack Ng,[2] Bernard Chern,[2] George SH Yeo,[3] Kok Hian Tan [2]

[1]MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[2]Division of Obstetrics and Gynaecology, KK Women's and Children's Hospital, Singapore

[3]Department of Maternal and Fetal Medicine, KK Women's and Children's Hospital, Singapore

Corresponding Author: Dr. Jun Zhang

Xinhua Hospital, Shanghai Jiao Tong University School of Medicine

No. 1665 Kongjiang Road, Shanghai, 200092, China

Telephone: 86-21-25078871

E-mail: junjimzhang@sina.com; zhangjun@xinhuamed.com.cn

Word count: abstract 285; main text 2492

Number of tables: 4

Number of figures: 1

Abstract

Objective

It remains unclear what roles placenta-originated angiogenic factors play in the pathogenesis of preeclampsia among hypertensive women. We compared maternal soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) levels throughout pregnancy in women with normal blood pressure (BP), elevated BP and hypertension in early pregnancy and their risks of developing hypertensive disorders.

Design

A prospective cohort study.

Setting

KK Women's and Children's Hospital, Singapore.

Participants

923 women with singleton pregnancy less than 14 weeks of gestation were included in the prospective Neonatal and Obstetrics Risks Assessment cohort between September 2010 and October 2014. Systolic, diastolic, mean arterial blood pressure (MAP) were measured at 11-14 weeks.

Primary and secondary outcomes

Maternal serum sFlt-1, PlGF and sFlt-1/PlGF ratio were tested at 11-14 weeks, 18-22 weeks, 28-32 weeks and 34 weeks onwards of gestation. Preeclampsia was main pregnancy outcome.

Results

Women were divided based on their BP in early pregnancy: normal (N=750), elevated BP (N=98) and hypertension (N=75). Maternal sFlt-1 levels and sFlt-1/PlGF ratios

were higher in hypertensive women throughout pregnancy, but maternal PlGF levels were not significantly lower. Rise in maternal systolic, diastolic BP and MAP at 11-14 weeks were significantly associated with higher sFlt-1/PlGF ratios during pregnancy. A 10 mmHg increase in MAP was associated with a 5.6-fold increase in risk of preterm preeclampsia and a 3.3-fold increase in risk of term preeclampsia, respectively.

Conclusion

Women with elevated blood pressure in early pregnancy already have a higher sFlt-1/PlGF ratio in early gestation and throughout pregnancy, and an increased risk of hypertensive complications. In contrast, PlGF levels in these women remain normal, suggesting that it is the vascular dysfunction, not the placenta, that plays a critical role in the pathogenesis of preeclampsia.

Key words

blood pressure, soluble fms-like tyrosine kinase 1, placental growth factor, preeclampsia

Strengths and limitations of the study

- This study was based on a well-performed perspective cohort with comprehensive information on clinical, biophysical and biochemical markers.
- Covariance analysis was performed to compare differences of angiogenic factors values among groups; multivariable logistic regression analysis was performed to evaluate the association between early pregnancy blood pressure and pregnancy outcomes.
- Given that most of our participants were low-risk pregnant women, our results may not be applicable to high-risk women.

Introduction

The imbalance in placenta-originated angiogenic factors has been found to play an important role in the pathogenesis of preeclampsia in recent years. Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) are the most studied proteins. sFlt-1, a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1, is a circulating anti-angiogenic protein that inhibits proangiogenic factors-VEGF and placental growth factor (PlGF) signaling in the vasculature.[1] High levels of circulating sFlt-1 and low levels of PlGF were observed in women with established preeclampsia and even before the onset of clinical symptoms. [2,3] These promising findings have been adopted and recommended by the National Institute for Clinical Excellence (NICE) to rule out preeclampsia in women presenting with clinical suspicion.[4]

Numerous studies focused on the pathological effects of excess sFlt-1 on endothelial function. It was observed that overexpression of sFlt-1 produced a preeclampsia-like syndrome of hypertension, proteinuria and glomerular endotheliosis in experimental animals.[5] sFlt-1 is largely made by syncytiotrophoblast and secreted into maternal circulation.[6] Placental hypoxia may be one of the main triggers of inducing abundant sFlt-1 expression and leading to hypertensive complications.[7,8] However, this hypothesis may not totally explain why women with elevated blood pressure before pregnancy have a substantially higher risk of preeclampsia. The objective of this study is to examine the dynamic changes of angiogenic and anti-angiogenic factors

throughout pregnancy in Asian women with elevated blood pressure in early gestation and their risks of developing hypertensive disorders later in pregnancy.

Methods

Study design

The Neonatal and Obstetrics Risks Assessment (NORA) study was a prospective cohort conducted at the KK Women's and Children's Hospital (KKH) in Singapore.[9] The cohort was set up to screen factors associated with adverse perinatal outcomes, with a focus on using clinical, biochemical and biophysical markers to predict the risks of pregnancy complications in early pregnancy. In brief, detailed interviews, ultrasound scans and blood sample collections were performed at recruitment (11 to 14 weeks), 18 to 22 weeks, 28 to 32 weeks and 34 weeks onward, respectively. All 4 antenatal visits also included measurement of maternal height and weight; recording of blood pressure by validated automated devices according to the recommendations of the American Heart Association.[10] Participants were closely followed up till their postnatal discharge from the hospital. Information on pregnancy complications, labor and delivery and neonatal outcomes was collected through medical chart review. The study protocol was approved by the SingHealth Centralised Institutional Review Board Ethics Committee, Singapore (CIRB Ref No. 2010/214/D), and a written informed consent was obtained from all participating women.

Study population

The NORA cohort recruited women with confirmed singleton pregnancies less than 14 weeks of gestation between September 2010 and October 2014. The exclusion criteria were multiple gestation, severe medical conditions such as chronic renal disease or systemic lupus erythematosus and pregnancies complicated by aneuploidy or fetal anomaly. Gestational age was confirmed by ultrasound at recruitment. A total of 1013 women were enrolled and 934 of them completed all 4 antenatal visits. 8 participants were delivered elsewhere, leaving 926 eligible women in the cohort. To evaluate the impacts of maternal blood pressure in early pregnancy on angiogenic factors levels and pregnancy outcomes, we used blood pressure at recruitment (11-14 weeks) to classify women into normal, elevated and hypertension groups. As 3 women did not have blood pressure records at recruitment (11-14 weeks), we included 923 participants for the final analysis (Figure 1).

Diagnosis

Preeclampsia was defined according to the guidelines of International Society for the Study of Hypertension in Pregnancy [11]: systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least two occasions four hours apart after 20 weeks of gestation in a previously normotensive women, and proteinuria: urinary albumin ≥300mg/24 hours urine collection or ≥1+ dipstick. We used the gestational age at delivery to divide cases of preeclampsia into term (≥37 weeks) and preterm term (< 37 weeks). Gestational hypertension was defined as newly onset hypertension after 20 weeks of gestation without proteinuria. Chronic

hypertension was defined as women with history of hypertension before conception or the presence of hypertension before 20 weeks of gestation.

To explore the relationship between maternal blood pressure levels in the first trimester and pregnancy outcome, we followed the 2017 guideline from the American College of Cardiology and the American Heart Association (ACC/AHA).[12] Normal blood pressure was defined as SBP < 120 mmHg and DBP < 80 mmHg; elevated blood pressure was defined as SBP 120-129 mmHg and DBP < 80 mmHg; hypertension stage 1 as SBP 130-139 mmHg or DBP 80-89 mmHg and hypertension stage 2 as SBP≥140 mmHg or DBP ≥90 mmHg.

Measurement of blood pressure

Blood pressure was taken by validated automated devices which were calibrated periodically. The women were in the seated position and their arms were supported. A correct cuff size was used and the middle of cuff was positioned on woman's upper arm at the level of the right atrium. After a five-minute rest, blood pressure was measured by trained nurses and three recordings were made at 1-minute intervals. We calculated SBP and DBP as the average of the three measurements. Mean arterial pressure (MAP) was calculated from SBP and DBP measures using the following formula: MAP=DBP+1/3×(SBP-DBP). Blood pressure was modeled continuously in units of 10 mm of mercury (mmHg).

Measurements of angiogenic factors

About 8 ml of maternal venous blood was collected in non-heparinised tubes at each antenatal visit. It was then centrifuged at 2000rpm for 15 minutes before separating and storing serum samples at -80°C for subsequent analysis. Serum levels of sFlt-1 and PlGF were determined by means of the fully automated Elecsys assays on an electrochemiluminescence immunoassay platform (cobas e411 analyzers, Roche Diagnostics). The detection limit was approximately 6 pg/ml for sFlt-1 and <2 pg/ml for PlGF.

Statistical analysis

Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. Numeric data were expressed as mean (standard deviation) or as median (interquartile range) for normally and non-normally distributed data, respectively. Maternal characteristics and pregnancy outcomes were compared among normal, elevated blood pressure and hypertension (stage 1 and stage 2) groups using one-way ANOVA or Kruskal-Wallis tests for continuous variables and χ^2 analysis for categorical variables. Covariance analysis and Dunnett test was used to compare differences of logarithm-transformed angiogenic factors values among groups adjusted for covariants. Geometric means and 95% confidence intervals were calculated by taking the exponent of the logarithm transformed mean. Linear regression analysis was performed to assess the association of blood pressure (10 mmHg) at recruitment with logarithm-transformed angiogenic factors values during pregnancy. Covariance

analysis and linear regression models were adjusted for maternal race, smoking during pregnancy, body mass index (BMI) and gestational age at blood collection as covariant. Logistic regression analysis was performed to evaluate the association between early pregnancy blood pressure (10 mmHg) and pregnancy outcomes with adjustment of potential confounders, including maternal age, race, education, maternal BMI at recruitment, chronic hypertension and preexisting diabetes mellitus. We used SAS version 9.4 (Cary, NC) for all statistical analyses.

Results

A total of 923 participants in the NORA cohort were included in this analysis. Based on maternal blood pressure at recruitment at 11-14 weeks of gestation, 750 women were classified as normal blood pressure; 98 women as elevated blood pressure, and 75 women as hypertension (stage 1 and stage 2). A comparison of maternal characteristics and pregnancy outcomes are given in Table 1. Women with hypertension (stage 1 and stage 2) in the first trimester were slightly older than the other two groups. Chinese women contributed a greater proportion of hypertension (49.3%) than Indian (8.0%) and Malay (30.7%). A higher incidence of maternal overweight (BMI ≥25 kg/m^2) and obesity (BMI ≥30 kg/m^2) was observed in the elevated blood pressure group and hypertension group than in the normal blood pressure group.

Table 1. Characteristics and pregnancy outcomes by maternal blood pressure at 11-14weeks in the NORA cohort.

Variables	Normal BP (n=750)	Elevated BP (n=98)	Hypertension (stage 1 and stage2) (n=75)	*P* value
Maternal age (year), median (IQR)	30.0 (26.0-34.0)	30.0 (26.0-35.0)	32.0 (29.0-35.0)	< 0.001
Race, n (%)				0.012
Chinese	393 (52.3)	38 (38.8)	37 (49.3)	
Indian	88 (11.7)	6 (6.1)	6 (8.0)	
Malay	186 (24.8)	41 (41.8)	23 (30.7)	
Others	84 (11.2)	13 (13.3)	9 (12.0)	
Parity, n (%)				0.091
0	419 (55.9)	49 (50.0)	33 (44.0)	
1	236 (31.5)	32 (32.7)	25 (33.3)	
≥ 2	95 (12.7)	17 (17.3)	17 (22.7)	
Maternal education levels, n (%)				0.044
Less than high school	166 (22.2)	28 (28.6)	25 (33.3)	
High school	299 (40.0)	44 (44.9)	23 (30.7)	
College and above	282 (37.8)	26 (26.5)	27 (36.0)	
Married, n (%)	699 (93.2)	90 (91.8)	72 (96.0)	0.544
Smoking during pregnancy, n (%)	19 (2.5)	4 (4.1)	0 (0)	0.230
Maternal BMI at 11-14 weeks of gestation (kg/m2), n (%)				< 0.001
< 18.5	62 (8.3)	2 (2.0)	0 (0)	
18.5-24.9	475 (63.5)	32 (32.7)	16 (21.3)	
25.0-29.9	162 (21.7)	35 (35.7)	32 (42.7)	
≥ 30.0	49 (6.6)	29 (29.6)	27 (36.0)	
Diabetes mellitus, n (%)	7 (0.9)	2 (2.0)	5 (6.7)	< 0.001
ART conception, n (%)	31 (4.1)	4 (4.1)	2 (2.7)	0.826
Antihypertensive medication, n (%)	0 (0)	0 (0)	3 (4.0)	< 0.001
Pregnancy outcomes				
Delivery age (weeks), median (IQR)	39.0 (38.1-39.7)	38.7 (38.0-39.6)	38.3 (37.6-39.1)	0.001
Birth weight (kg), median (IQR)	3.1 (2.9-3.4)	3.1 (2.9-3.5)	3.1 (2.8-3.4)	0.324
Gestational hypertension, n (%)	10 (1.3)	3 (3.1)	5 (6.7)	0.004
Preeclampsia, n (%)	6 (0.8)	3 (3.1)	12 (16.0)	< 0.001

Preterm preeclampsia, n (%)	4 (0.5)	0 (0)	5 (6.7)	< 0.001
Term preeclampsia, n (%)	2 (0.3)	3 (3.1)	7 (9.3)	< 0.001

IQR: interquartile range; BMI: body mass index; ART: assisted reproductive technology; BP: blood pressure.

The prevalence of maternal preconception diabetes was 6.7% in hypertension groups which was significantly higher than that of the other two groups. However, the prevalence of conception with assisted reproductive technology (ART) were not significantly different among the three groups. As expected, the incidence of gestational hypertension (6.7%), preeclampsia (16.0%), preterm preeclampsia (6.7%) and term preeclampsia (9.3%) were the highest in the hypertension group. Women with hypertension had sustainable higher blood pressure levels during pregnancy (Table 2).

Table 2. Maternal blood pressure levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Variables	Normal BP (n=750)	Elevated BP (n=98)	Hypertension (stage 1 and stage2) (n=75)	*P* value
SBP (mmHg), mean±SD				
11-14 weeks	104.9±8.3	123.2±2.6	126.8±11.1	< 0.001
18-22 weeks	105.9±10.1	118.6±9.9	124.0±10.5	< 0.001
28-32 weeks	108.2±10.0	118.1±9.7	123.8±11.4	< 0.001
≥34 weeks	110.5±10.7	119.5±10.6	126.1±15.5	< 0.001
DBP (mmHg), mean±SD				
11-14 weeks	63.6±6.8	71.4±4.4	80.8±7.0	< 0.001

18-22 weeks	62.9±7.0	69.0±7.2	76.3±8.1	< 0.001
28-32 weeks	64.0±6.9	69.8±6.6	76.6±9.0	< 0.001
≥34 weeks	67.4±7.8	73.3±7.3	80.0±11.4	< 0.001
MAP (mmHg), mean±SD				
11-14 weeks	77.3±6.5	88.7±3.2	96.1±6.5	< 0.001
18-22 weeks	77.2±7.2	85.5±7.3	92.2±8.2	< 0.001
28-32 weeks	78.8±7.1	85.9±6.3	92.4±9.1	< 0.001
≥34 weeks	81.8±8.1	88.7±7.6	95.4±11.9	< 0.001

BP: blood pressure; SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure

Table 3 shows the values of angiogenic factors at 4 time points in the NORA participants. We used covariance analysis to control for potential confounders that were reported to have impact on serum angiogenic proteins levels, including maternal race, smoking, maternal BMI and gestational age at blood collection. Overall, serum sFlt-1 concentrations continued rising throughout pregnancy. PlGF levels increased from the first trimester, peaked at 28-32 weeks and declined afterwards. Consequently, high levels of sFlt-1/PlGF ratio were observed both at 11-14 weeks and 34 weeks onwards. The dynamic change of serum angiogenic factors during pregnancy was observed in all 3 groups. Maternal serum sFlt-1 and PlGF levels were not significantly different between elevated BP group and normal BP group at 4 time points during pregnancy. In hypertension group, a trend of higher maternal sFlt-1 concentrations was observed from early pregnancy and it was dramatically increased

during the third trimester compared with sFlt-1 levels in normotensive women. In contrast, PlGF concentrations were not significantly different between hypertension group and normal BP group. Thus, sFlt-1/PlGF ratio in hypertensive women was significantly higher throughout pregnancy than that in normotensive women.

Table 3. Maternal serum angiogenic factors levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Angiogenic factors	Time points	Normal BP		Elevated BP			Hypertension (stage 1 and stage 2)		
		N	mean (95%CI) *	N	mean (95%CI) *	*P* value †	N	mean (95%CI) *	*P* value ‡
sFlt-1 (pg/ml)	11-14 weeks	746	1585 (1549, 1622)	98	1722 (1585, 1862)	0.096	75	1758 (1585, 1950)	0.079
	18-22 weeks	745	1698 (1660, 1778)	98	1738 (1585, 1950)	0.824	75	1905 (1698, 2138)	0.139
	28-32 weeks	730	1660 (1585, 1698)	92	1698 (1549, 1862)	0.868	70	2042 (1820, 2291)	0.001
	≥34 weeks	659	2570 (2512, 2692)	82	2818 (2570, 3162)	0.201	57	3311 (2951, 3802)	0.000
PlGF (pg/ml)	11-14 weeks	746	37 (36, 38)	98	35 (32, 38)	0.243	75	35 (32, 39)	0.593
	18-22 weeks	745	269 (257, 275)	98	245 (224, 269)	0.261	75	245 (219, 275)	0.364
	28-32 weeks	730	617 (589, 646)	92	575 (501, 676)	0.718	70	537 (457, 631)	0.270
	≥34 weeks	659	380 (355, 398)	82	324 (269, 380)	0.178	57	339 (275, 427)	0.585
sFlt-1/ PlGF ratio	11-14 weeks	746	42.7 (40.7, 44.7)	98	50.1 (44.7, 55.0)	0.013	75	50.1 (43.7, 56.2)	0.042
	18-22 weeks	745	6.5 (6.2, 6.8)	98	7.1 (6.3, 7.9)	0.201	75	7.8 (6.8, 8.9)	0.027
	28-32 weeks	730	2.7 (2.5, 2.9)	92	2.9 (2.5, 3.5)	0.643	70	3.8 (3.1, 4.6)	0.005
	≥34 weeks	659	6.9 (6.3, 7.4)	82	8.9 (6.9, 11.2)	0.100	57	9.8 (7.4, 13.2)	0.044

BP, blood pressure; CI, confidence interval.

*Means (95%CI) are adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes; presented as geometric means.

†statistically significant difference between normal BP and elevated BP groups.

‡statistically significant difference between normal BP and hypertension group.

Higher levels of DBP and MAP in early pregnancy were significantly associated with higher log-transformed sFlt-1 values throughout pregnancy. Meanwhile, higher SBP levels were significantly associated with lower log-transformed PlGF levels both at 18-22 weeks (β= -0.02 per 10 mmHg SBP, *P*=0.011) and at 28-32 weeks (β= -0.02 per 10 mmHg SBP, *P*=0.031). Thus, rises in maternal SBP, DBP and MAP in the first trimester were significantly associated with higher sFlt-1/PlGF ratios during pregnancy (supplementary table 1).

Table 4 presents the significant association between blood pressure in early pregnancy and risks of preeclampsia (OR 2.5, 95% CI 1.5-4.0 per 10 mmHg SBP; OR 4.3, 95% CI 2.3-7.9 per 10 mmHg DBP; OR 4.1, 95% CI 2.2-7.7 per 10 mmHg MAP, respectively) after adjustment of potential confounders. Preterm preeclampsia was more closely associated with higher DBP than SBP (OR 6.0, 95% CI 2.3-7.9 per 10 mmHg DBP *vs.* OR 1.9, 95% CI 0.9-3.8 per 10 mmHg SBP).

Table 4. Logistic regression analysis for maternal blood pressure at 11-14weeks and adverse pregnancy outcomes.

Variable	Preeclampsia		Preterm preeclampsia		Term preeclampsia	
	Crude OR	Adjusted OR* (95% CI)	Crude OR	Adjusted OR* (95% CI)	Crude OR	Adjusted OR* (95% CI)
SBP (10mmHg)	3.0	2.5 (1.5, 4.0) †	1.9	1.9 (0.9, 3.8)	4.1	3.2 (1.6, 6.4) †
DBP (10mmHg)	5.2	4.3 (2.3, 7.9) †	5.8	6.0 (2.3, 16.2) †	4.4	3.1 (1.4, 6.8) †
MAP (10mmHg)	5.1	4.1 (2.2, 7.7) †	5.1	5.6 (2.0, 15.5) †	4.7	3.3 (1.5, 7.4) †

OR, odds ratio; CI, confidence interval; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure

*Adjusted for maternal age, race, education, maternal body mass index at 11-14 weeks gestation and diabetes mellitus.

†P < 0.01.

Discussion

Our study confirms that higher early pregnancy blood pressure levels are prospectively associated with increased risks of preeclampsia, including preterm and term preeclampsia. Furthermore, women with elevated blood pressure in early pregnancy already had a higher sFlt-1 level and sFlt-1/PlGF ratio in early gestation and throughout pregnancy. In contrast, PlGF levels in these women remained normal throughout gestation.

It is well established that women with chronic hypertension have several times the risk of preeclampsia than normotensive women.[13] However, the pathogensis is poorly understood and what role these angiogenic and anti-angiogenic factors play remains unclear. Although syncytiotrophoblast is a major source of sFlt-1 production, peripheral blood monocytes produce a small amount of sFlt-1 further stimulated by inflammation.[14,15] As chronic hypertension is often related to a chronic inflammatory status,[16] the slightly increased sFlt-1 level in hypertensive women in early pregnancy, as observed in our study, may reflect the chronic inflammatory status in early pregnancy. Our results suggest an imbalanced angiogenic environment in early pregnancy along with chronic inflammation may injure maternal vascular function and cause a preexisting maternal endothelial dysfunction. The latter, in turn, increases the risk of hypertensive disorders in pregnancy.

On the other hand, maternal circulating PlGF is highly expressed during pregnancy by the placenta. It has both vasculogenic and angiogenic functions [17] and its level is likely to reflect the placental health conditions. For example, low PlGF concentrations preceding clinical onset of preeclampsia often occur in early-onset rather than late-onset preeclampsia.[18] Women who develop preeclampsia with fetal growth restriction (FGR) have further decreased PlGF levels compared with women who develop preeclampsia without FGR.[19-21] Early-onset preeclampsia and placenta-derived FGR are associated with placenta pathology including incomplete remodeling of spiral arteries, acute atherosis and thrombosis in spiral arteries and syncytiotrophoblast necrosis.[22,23] In our study, women with elevated blood pressure in early pregnancy, the PlGF level remained by and large normal throughout pregnancy and newborns' birth weight was not significantly different among the three groups, suggesting that the placental implantation and development may not be impaired in these women.

As poor placentation is not a unique cause of developing preeclampsia, enhanced placental oxidative and endoplasmic reticulum stress and increased maternal systemic inflammatory responses are thought to play crucial roles in preeclampsia as well.[24,25] Thus, preexisting endothelial dysfunction in hypertensive women could be exacerbated as a result of physiological burden of pregnancy even without an abnormal placentation.[26] Taking all things considered, we propose that the imbalanced angiogenic factors environment and, perhaps more importantly, preexisting endothelial susceptibility and dysfunction, may play a critical role in the development

of preeclampsia in women with elevated blood pressure in early pregnancy.

Our study was a well-performed prospective study with comprehensive information including clinical, biophysical and biochemical markers. The follow-up rate was 99.1% (926/934) at the end of pregnancy. Measurements of blood pressure and serum angiogenic factors were performed according to standardized protocols. In our study, three women reported using antihypertensive medications during pregnancy; thus, the results should not be affected by the medication issue. On the other hand, as most of our participants were low-risk pregnant women, our results may not be applicable to high-risk women.

Conclusion

Women with elevated blood pressure in early pregnancy already have a higher sFlt-1/PlGF ratio in early gestation and throughout pregnancy, and an increased risk of hypertensive complications. In contrast, PlGF levels in these women remain normal throughout gestation, suggesting that it is the vascular dysfunction, not the placenta, that plays a critical role in the pathogenesis of preeclampsia. Our study also supports that preconception or early pregnancy high blood pressure, defined as SBP≥130 mmHg or DBP≥80 mmHg according to 2017 ACC/AHA guideline, should cause clinical awareness both during pregnancy and in their later life.

Acknowledgments

We thank all the NORA cohort (Neonatal and Obstetrics Risks Assessment) participants for their commitment to the study, the NORA cohort investigators and staff.

Author Contributions

JZ: performed the statistical analysis, searched literature and drafted the manuscript. JZ (corresponding author): had the original idea, provided guidance for the statistical analysis and revised the manuscript. MJN, BC, GSY and KHT: participated in the data collection, reviewed and revised the manuscript.

Funding: The authors received no finical support for the research, authorship, and/or publication of this article.

Competing interests: None declared.

References

1. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A* 1993; 90:10705-10709.
2. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, *et al*. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; 350(7): 672-83.
3. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, *et al.* Predictive Value of the sFlt-1: PlGF Ratio in Women with Suspected Preeclampsia. N E*ngl J Med* 2016 Jan 7;374(1):13-22.
4. NICE. PlGF-based Testing to Help Diagnose Suspected Pre-eclampsia (Triage PlGF Test, Elecsys Immunoassay sFlt-1/PlGF Ratio, DELFIA Xpress PlGF 1-2-3 Test, and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio). Diagnostics Guidance 23. London: NICE, 2016.
5. 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. *J Clin Invest* 2003;111(5):649-58.
6. Sela S, Itin A, Natanson-Yaron S, Greenfield C, Goldman-Wohl D, Yagel S, *et al.* A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-type-specific splicing and implications to vascular endothelial growth factor homeostasis and preeclampsia. *Circ Res* 2008;102(12):1566-74.
7. Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem* 1997;272(38):23659-67.
8. Warrington JP, George EM, Palei AC, Spradley FT, Granger JP. Recent advances in the understanding of the pathophysiology of preeclampsia. *Hypertension* 2013;62(4):666-73.
9. Ng QJ, Zhang J, Dai F, Ng MJ, Razali NS, Win NM, *et al.* Neonatal and Obstetric

Risk Assessment (NORA) Pregnancy Cohort Study in Singapore. *Int J Gynaecol Obstet* 2018; 4(1): 31-37.

10. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, *et al*. Recommendations for blood pressure measurement in humans and experimental animals. *Circulation* 2005; 111:697-716.
11. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; 20(1): IX-XIV.
12. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, *et al.* 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018;71(6):1269-1324.
13. Bartsch E, Medcalf KE, Park AL, Ray JG; High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. *BMJ* 2016;353: i1753.
14. Rajakumar A, Michael HM, Rajakumar PA, Shibata E, Hubel CA, Karumanchi SA, *et al.* Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. *Placenta* 2005;26(7):563-73.
15. Freeman DJ, McManus F, Brown EA, Cherry L, Norrie J, Ramsay JE, *et al.* Short- and long-term changes in plasma inflammatory markers associated with preeclampsia. *Hypertension* 2004;44(5):708-14.
16. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *Biomed Res Int* 2014;

2014: 406960.

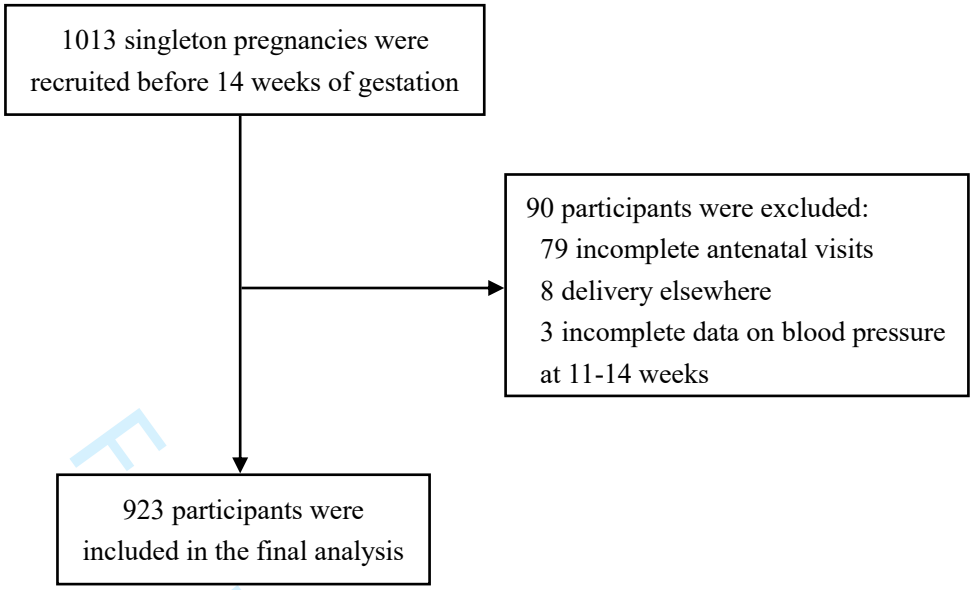
17. De Falco S. The discovery of placenta growth factor and its biological activity. *Exp Mol Med* 2012;44(1):1-9.
18. McElrath TF, Lim KH, Pare E, Rich-Edwards J, Pucci D, Troisi R, *et al.* Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. *Am J Obstet Gynecol* 2012;207(5): 407.e1-7.
19. Powers RW, Roberts JM, Plymire DA, Pucci D, Datwyler SA, Laird DM, *et al.* Low placental growth factor across pregnancy identifies a subset of women with preterm preeclampsia: type 1 versus type 2 preeclampsia? *Hypertension* 2012;60(1):239-46.
20. 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. *J Matern Fetal Neonatal Med* 2008;21(1):9-23.
21. Chaiworapongsa T, Romero R, Whitten AE, Korzeniewski SJ, Chaemsaithong P, Hernandez-Andrade E, *et al.* The use of angiogenic biomarkers in maternal blood to identify which SGA fetuses will require a preterm delivery and mothers who will develop pre-eclampsia. *J Matern Fetal Neonatal Med* 2016;29(8):1214-28.
22. Nelson DB, Ziadie MS, McIntire DD, Rogers BB, Leveno KJ. Placental pathology suggesting that preeclampsia is more than one disease. *Am J Obstet Gynecol* 2014;210(1): 66.e1-7.
23. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. *Am J Obstet Gyneco.* 2018;218(2S): S745-S761.
24. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet* 2010;376(9741):631-44.
25. Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? *Placenta* 2014;35 Suppl:

S20-5.

26. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am J Obstet Gynecol* 1996;175(5):1365-70.

Figure legends

Figure 1. Flowchart of participants.



Supplementary table 1. Associations of maternal blood pressure at 11-14weeks with serum angiogenic factors levels during pregnancy.

Variable	Time points	Log sFlt-1		Log PlGF		Log sFlt-1/ PlGF ratio	
		β (95% CI) *	*P* value	β (95% CI) *	*P* value	β (95% CI) *	*P* value
Systolic blood pressure (10mmHg)	11-14 weeks	0.01 (0.004, 0.03)	0.009	-0.01 (-0.02, 0.0004)	0.057	0.03 (0.01, 0.04)	< 0.001
	18-22 weeks	0.01 (-0.01, 0.02)	0.227	-0.02 (-0.03, -0.004)	0.011	0.02 (0.01, 0.04)	0.003
	28-32 weeks	0.01 (-0.001, 0.02)	0.068	-0.02 (-0.04, -0.002)	0.031	0.03 (0.01, 0.06)	0.007
	≥34 weeks	0.02 (0.01, 0.03)	0.009	-0.02 (-0.05, 0.001)	0.059	0.04 (0.01, 0.07)	0.011
Diastolic blood pressure (10mmHg)	11-14 weeks	0.02 (0.01, 0.04)	0.002	-0.01 (-0.03, 0.001)	0.069	0.04 (0.02, 0.05)	< 0.001
	18-22 weeks	0.02 (0.004, 0.04)	0.014	-0.01 (-0.02, 0.01)	0.388	0.03 (0.01, 0.05)	0.008
	28-32 weeks	0.04 (0.02, 0.05)	< 0.001	-0.02 (-0.04, 0.004)	0.104	0.05 (0.03, 0.08)	< 0.001
	≥34 weeks	0.04 (0.02, 0.06)	< 0.001	-0.02 (-0.05, 0.01)	0.134	0.06 (0.02, 0.10)	0.003
Mean arterial pressure (10mmHg)	11-14 weeks	0.02 (0.01, 0.04)	0.002	-0.02 (-0.03, -0.001)	0.043	0.04 (0.02, 0.06)	< 0.001
	18-22 weeks	0.02 (0.001, 0.03)	0.044	-0.01 (-0.02, 0.01)	0.506	0.02 (0.002, 0.04)	0.030
	28-32 weeks	0.03 (0.01, 0.04)	0.001	-0.02 (-0.05, 0.001)	0.058	0.05 (0.02, 0.08)	0.001
	≥34 weeks	0.04 (0.02, 0.05)	< 0.001	-0.03 (-0.06, -0.003)	0.029	0.07 (0.03, 0.11)	< 0.001

Abbreviations: CI, confidence interval.

*Adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test.

BMJ Open

Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032237.R1
Article Type:	Original research
Date Submitted by the Author:	18-Sep-2019
Complete List of Authors:	Zhu, Jing; MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China Zhang, Jun; Ministry of Education–Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine Ng, Mor Jack; KK Women's and Children's Hospital, Division of Obstetrics and Gynaecology Chern, Bernard; KK Women's and Children's Hospital Yeo, George S. H.; KK Women's and Children's Hospital, Singapore Tan, Kok Hian; KK Women's and Children's Hospital, Division of Obstetrics and Gyneacology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Epidemiology
Keywords:	blood pressure, soluble fms-like tyrosine kinase 1, placental growth factor, preeclampsia

Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study

Jing Zhu,[1] Jun Zhang,[1,2] Mor Jack Ng,[2] Bernard Chern,[2] George SH Yeo,[3] Kok Hian Tan [2]

[1]MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[2]Division of Obstetrics and Gynaecology, KK Women's and Children's Hospital, Singapore

[3]Department of Maternal and Fetal Medicine, KK Women's and Children's Hospital, Singapore

Corresponding Author: Dr. Jun Zhang

Xinhua Hospital, Shanghai Jiao Tong University School of Medicine

No. 1665 Kongjiang Road, Shanghai, 200092, China

Telephone: 86-21-25078871

E-mail: junjimzhang@sina.com; zhangjun@xinhuamed.com.cn

Word count: abstract 262; main text 2525

Number of tables: 4

Number of figures: 1

Abstract

Objective

It remains unclear what roles placenta-originated angiogenic factors play in the pathogenesis of preeclampsia among hypertensive women. We compared maternal soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) levels throughout pregnancy in women with normal blood pressure (BP), elevated BP and hypertension in early pregnancy and their risks of developing preeclampsia.

Design

A prospective cohort study.

Setting

KK Women's and Children's Hospital, Singapore.

Participants

923 women with singleton pregnancy less than 14 weeks of gestation were included in the prospective Neonatal and Obstetrics Risks Assessment cohort between September 2010 and October 2014. Systolic, diastolic, mean arterial blood pressure (MAP) were measured at 11-14 weeks.

Primary and secondary outcomes

Maternal serum sFlt-1, PlGF and sFlt-1/PlGF ratio were tested at 11-14 weeks, 18-22 weeks, 28-32 weeks and 34 weeks onwards of gestation. Preeclampsia was main pregnancy outcome.

Results

Women were divided based on their BP in early pregnancy: normal (N=750), elevated BP (N=98) and hypertension (N=75). Maternal sFlt-1 levels and sFlt-1/PlGF ratios

were higher in hypertensive women throughout pregnancy, but maternal PlGF levels were not significantly lower. Rise in maternal systolic, diastolic BP and MAP at 11-14 weeks were significantly associated with higher sFlt-1/PlGF ratios during pregnancy. A 10mmHg increase in MAP was associated with a 5.6-fold increase in risk of preterm preeclampsia and a 3.3-fold increase in risk of term preeclampsia, respectively.

Conclusion

Women with elevated blood pressure in early pregnancy already had a higher sFlt-1/PlGF ratio in early gestation and throughout pregnancy, and an increased risk of preeclampsia. In contrast, PlGF levels in these women remained normal.

Key words

blood pressure, soluble fms-like tyrosine kinase 1, placental growth factor, preeclampsia

Strengths and limitations of the study

- This study was based on a well-performed perspective cohort with comprehensive information on clinical, biophysical and biochemical markers.
- Covariance analysis was performed to compare differences of angiogenic factors values among groups; multivariable logistic regression analysis was performed to evaluate the association between early pregnancy blood pressure and pregnancy outcomes.
- Given that most of our participants were low-risk pregnant women, our results may not be applicable to high-risk women.

Introduction

The imbalance in placenta-originated angiogenic factors has been found to play an important role in the pathogenesis of preeclampsia in recent years. Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) are the most studied proteins. sFlt-1, a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1, is a circulating anti-angiogenic protein that inhibits proangiogenic factors-VEGF and placental growth factor (PlGF) signaling in the vasculature.[1] High levels of circulating sFlt-1 and low levels of PlGF were observed in women with established preeclampsia and even before the onset of clinical symptoms. [2,3] These promising findings have been adopted and recommended by the National Institute for Clinical Excellence (NICE) to rule out preeclampsia in women presenting with clinical suspicion.[4]

Numerous studies focused on the pathological effects of excess sFlt-1 on endothelial function. It was observed that overexpression of sFlt-1 produced a preeclampsia-like syndrome of hypertension, proteinuria and glomerular endotheliosis in experimental animals.[5] sFlt-1 is largely produced by syncytiotrophoblast and secreted into maternal circulation.[6] Placental hypoxia may be one of the main triggers of inducing abundant sFlt-1 expression and leading to hypertensive complications.[7,8] However, this hypothesis may not totally explain why women with elevated blood pressure before pregnancy have a substantially higher risk of preeclampsia. Besides, evidence suggests that there might be some racial differences in maternal angiogenic and anti-

angiogenic factors. [9] Thus, the objective of this study is to examine the dynamic changes of angiogenic and anti-angiogenic factors throughout pregnancy in Asian women with elevated blood pressure in early gestation and their risks of developing preeclampsia later in pregnancy.

Methods

Study design

The Neonatal and Obstetrics Risks Assessment (NORA) study was a prospective cohort conducted at the KK Women's and Children's Hospital (KKH) in Singapore.[10] The cohort was set up to screen factors associated with adverse perinatal outcomes, with a focus on using clinical, biochemical and biophysical markers to predict the risks of pregnancy complications in early pregnancy. In brief, detailed interviews, ultrasound scans and blood sample collections were performed at recruitment (11 to 14 weeks), 18 to 22 weeks, 28 to 32 weeks and 34 weeks onward, respectively. All 4 antenatal visits also included measurement of maternal height and weight; recording of blood pressure by validated automated devices according to the recommendations of the American Heart Association.[11] Participants were closely followed up till their postnatal discharge from the hospital. Information on pregnancy complications, labor and delivery and neonatal outcomes was collected through medical chart review. The study protocol was approved by the SingHealth Centralised Institutional Review Board Ethics Committee, Singapore (CIRB Ref No. 2010/214/D), and a written informed consent was obtained from all participating women.

Study population

The NORA cohort recruited women with confirmed singleton pregnancies less than 14 weeks of gestation between September 2010 and October 2014. The exclusion criteria were multiple gestation, severe medical conditions such as chronic renal disease or systemic lupus erythematosus and pregnancies complicated by aneuploidy or fetal anomaly. Gestational age was confirmed by ultrasound at recruitment. A total of 1013 women were enrolled and 934 of them completed all 4 antenatal visits. 8 participants were delivered elsewhere, leaving 926 eligible women in the cohort. To evaluate the impacts of maternal blood pressure in early pregnancy on angiogenic factors levels and pregnancy outcomes, we used blood pressure at recruitment (11-14 weeks) to classify women into normal, elevated and hypertension groups. As 3 women did not have blood pressure records at recruitment (11-14 weeks), we included 923 participants for the final analysis (Figure 1).

Diagnosis

Preeclampsia was defined according to the guidelines of International Society for the Study of Hypertension in Pregnancy [12]: systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least two occasions four hours apart after 20 weeks of gestation in a previously normotensive women, and proteinuria: urinary albumin ≥300mg/24 hours urine collection or ≥1+ dipstick. We used the gestational age at delivery to divide cases of preeclampsia into term (≥37

weeks) and preterm term (< 37 weeks). Gestational hypertension was defined as newly onset hypertension after 20 weeks of gestation without proteinuria. Chronic hypertension was defined as women with history of hypertension before conception or the presence of hypertension before 20 weeks of gestation.

To explore the relationship between maternal blood pressure levels in the first trimester and pregnancy outcome, we followed the 2017 guideline from the American College of Cardiology and the American Heart Association (ACC/AHA).[13] Normal blood pressure was defined as SBP < 120 mmHg and DBP < 80 mmHg; elevated blood pressure was defined as SBP 120-129 mmHg and DBP < 80 mmHg; hypertension stage 1 as SBP 130-139 mmHg or DBP 80-89 mmHg and hypertension stage 2 as SBP≥140 mmHg or DBP ≥90 mmHg.

Measurement of blood pressure

Blood pressure was taken by validated automated devices which were calibrated periodically. The women were in the seated position and their arms were supported. A correct cuff size was used and the middle of cuff was positioned on woman's upper arm at the level of the right atrium. After a five-minute rest, blood pressure was measured by trained nurses and three recordings were made at 1-minute intervals. We calculated SBP and DBP as the average of the three measurements. Mean arterial pressure (MAP) was calculated from SBP and DBP measures using the following formula: MAP=DBP+1/3×(SBP-DBP). Blood pressure was modeled continuously in

units of 10 mm of mercury (mmHg).

Measurements of angiogenic factors

About 8 ml of maternal venous blood was collected in non-heparinised tubes at each antenatal visit. It was then centrifuged at 2000rpm for 15 minutes before separating and storing serum samples at -80°C for subsequent analysis. Serum levels of sFlt-1 and PlGF were determined by means of the fully automated Elecsys assays on an electrochemiluminescence immunoassay platform (cobas e411 analyzers, Roche Diagnostics). The detection limit was approximately 6 pg/ml for sFlt-1 and <2 pg/ml for PlGF.

Statistical analysis

Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. Numeric data were expressed as mean (standard deviation) or as median (interquartile range) for normally and non-normally distributed data, respectively. Maternal characteristics, pregnancy outcomes and maternal blood pressure levels were compared among normal, elevated blood pressure and hypertension (stage 1 and stage 2) groups using one-way ANOVA or Kruskal-Wallis tests for continuous variables and χ^2 analysis for categorical variables. Covariance analysis and Dunnett test was used to compare differences of logarithm-transformed angiogenic factors values among groups adjusted for covariants. Geometric means and 95% confidence intervals were calculated by taking the exponent of the logarithm transformed mean.

Linear regression analysis was performed to assess the association of blood pressure (10 mmHg) at recruitment with logarithm-transformed angiogenic factors values during pregnancy. Covariance analysis and linear regression models were adjusted for maternal race, smoking during pregnancy, body mass index (BMI) and gestational age at blood collection as covariant. Logistic regression analysis was performed to evaluate the association between early pregnancy blood pressure (10 mmHg) and pregnancy outcomes with adjustment of potential confounders, including maternal age, race, education, maternal BMI at recruitment, chronic hypertension and preexisting diabetes mellitus. We used SAS version 9.4 (Cary, NC) for all statistical analyses.

Patient and public involvement

Patients and the public were not directly involved in the design, conduct or reporting in our study.

Results

A total of 923 participants in the NORA cohort were included in this analysis. Based on maternal blood pressure at recruitment at 11-14 weeks of gestation, 750 women were classified as normal blood pressure; 98 women as elevated blood pressure, and 75 women as hypertension (stage 1 and stage 2). A comparison of maternal characteristics and pregnancy outcomes are given in Table 1. Women with hypertension (stage 1 and stage 2) in the first trimester were slightly older than the

other two groups. Chinese women contributed a greater proportion of hypertension (49.3%) than Indian (8.0%) and Malay (30.7%). A higher incidence of maternal overweight (BMI ≥25 kg/m^2) and obesity (BMI ≥30 kg/m^2) was observed in the elevated blood pressure group and hypertension group than in the normal blood pressure group.

Table 1. Characteristics and pregnancy outcomes by maternal blood pressure at 11-14weeks in the NORA cohort.

Variables	Normal BP (n=750)	Elevated BP (n=98)	Hypertension (stage 1 and stage2) (n=75)	*P* value
Maternal age (year), median (IQR)	30.0 (26.0-34.0)	30.0 (26.0-35.0)	32.0 (29.0-35.0)	< 0.001
Race, n (%)				0.012
Chinese	393 (52.3)	38 (38.8)	37 (49.3)	
Indian	88 (11.7)	6 (6.1)	6 (8.0)	
Malay	186 (24.8)	41 (41.8)	23 (30.7)	
Others	84 (11.2)	13 (13.3)	9 (12.0)	
Parity, n (%)				0.091
0	419 (55.9)	49 (50.0)	33 (44.0)	
1	236 (31.5)	32 (32.7)	25 (33.3)	
≥ 2	95 (12.7)	17 (17.3)	17 (22.7)	
Maternal education levels, n (%)				0.044
Less than high school	166 (22.2)	28 (28.6)	25 (33.3)	
High school	299 (40.0)	44 (44.9)	23 (30.7)	
College and above	282 (37.8)	26 (26.5)	27 (36.0)	
Married, n (%)	699 (93.2)	90 (91.8)	72 (96.0)	0.544
Smoking during pregnancy, n (%)	19 (2.5)	4 (4.1)	0 (0)	0.230
Maternal BMI at 11-14 weeks of gestation (kg/m2), n (%)				< 0.001
< 18.5	62 (8.3)	2 (2.0)	0 (0)	
18.5-24.9	475 (63.5)	32 (32.7)	16 (21.3)	
25.0-29.9	162 (21.7)	35 (35.7)	32 (42.7)	
≥ 30.0	49 (6.6)	29 (29.6)	27 (36.0)	

Diabetes mellitus, n (%)	7 (0.9)	2 (2.0)	5 (6.7)	< 0.001
ART conception, n (%)	31 (4.1)	4 (4.1)	2 (2.7)	0.826
Antihypertensive medication, n (%)	0 (0)	0 (0)	3 (4.0)	< 0.001
Pregnancy outcomes				
Delivery age (weeks), median (IQR)	39.0 (38.1-39.7)	38.7 (38.0-39.6)	38.3 (37.6-39.1)	0.001
Birth weight (kg), median (IQR)	3.1 (2.9-3.4)	3.1 (2.9-3.5)	3.1 (2.8-3.4)	0.324
Gestational hypertension, n (%)	10 (1.3)	3 (3.1)	5 (6.7)	0.004
Preeclampsia, n (%)	6 (0.8)	3 (3.1)	12 (16.0)	< 0.001
Preterm preeclampsia, n (%)	4 (0.5)	0 (0)	5 (6.7)	< 0.001
Term preeclampsia, n (%)	2 (0.3)	3 (3.1)	7 (9.3)	< 0.001

IQR: interquartile range; BMI: body mass index; ART: assisted reproductive technology; BP: blood pressure.

The prevalence of maternal preconception diabetes was 6.7% in hypertension groups which was significantly higher than that of the other two groups. However, the prevalence of conception with assisted reproductive technology (ART) were not significantly different among the three groups. As expected, the incidence of gestational hypertension (6.7%), preeclampsia (16.0%), preterm preeclampsia (6.7%) and term preeclampsia (9.3%) were the highest in the hypertension group. Women with hypertension had sustainable higher blood pressure levels during pregnancy (Table 2).

Table 2. Maternal blood pressure levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Variables	Normal BP (n=750)	Elevated BP (n=98)	Hypertension (stage 1 and stage2) (n=75)	*P* value
SBP (mmHg), mean±SD				

11-14 weeks	104.9±8.3	123.2±2.6	126.8±11.1	< 0.001
18-22 weeks	105.9±10.1	118.6±9.9	124.0±10.5	< 0.001
28-32 weeks	108.2±10.0	118.1±9.7	123.8±11.4	< 0.001
≥34 weeks	110.5±10.7	119.5±10.6	126.1±15.5	< 0.001
DBP (mmHg), mean±SD				
11-14 weeks	63.6±6.8	71.4±4.4	80.8±7.0	< 0.001
18-22 weeks	62.9±7.0	69.0±7.2	76.3±8.1	< 0.001
28-32 weeks	64.0±6.9	69.8±6.6	76.6±9.0	< 0.001
≥34 weeks	67.4±7.8	73.3±7.3	80.0±11.4	< 0.001
MAP (mmHg), mean±SD				
11-14 weeks	77.3±6.5	88.7±3.2	96.1±6.5	< 0.001
18-22 weeks	77.2±7.2	85.5±7.3	92.2±8.2	< 0.001
28-32 weeks	78.8±7.1	85.9±6.3	92.4±9.1	< 0.001
≥34 weeks	81.8±8.1	88.7±7.6	95.4±11.9	< 0.001

BP: blood pressure; SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure

Table 3 shows the values of angiogenic factors at 4 time points in the NORA participants. We used covariance analysis to control for potential confounders that were reported to have impact on serum angiogenic proteins levels, including maternal race, smoking, maternal BMI and gestational age at blood collection. Overall, serum sFlt-1 concentrations continued rising throughout pregnancy. PlGF levels increased from the first trimester, peaked at 28-32 weeks and declined afterwards.

Consequently, high levels of sFlt-1/PlGF ratio were observed both at 11-14 weeks and 34 weeks onwards. The dynamic change of serum angiogenic factors during pregnancy was observed in all 3 groups. Maternal serum sFlt-1 and PlGF levels were not significantly different between elevated BP group and normal BP group at 4 time points during pregnancy. In hypertension group, a trend of higher maternal sFlt-1 concentrations was observed from early pregnancy and it was dramatically increased during the third trimester compared with sFlt-1 levels in normotensive women. In contrast, PlGF concentrations were not significantly different between hypertension group and normal BP group. Thus, sFlt-1/PlGF ratio in hypertensive women was significantly higher throughout pregnancy than that in normotensive women.

Table 3. Maternal serum angiogenic factors levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Angiogenic factors	Time points	Normal BP		Elevated BP			Hypertension (stage 1 and stage 2)		
		N	mean (95%CI) *	N	mean (95%CI) *	*P* value †	N	mean (95%CI) *	*P* value ‡
sFlt-1 (pg/ml)	11-14 weeks	746	1585 (1549, 1622)	98	1722 (1585, 1862)	0.096	75	1758 (1585, 1950)	0.079
	18-22 weeks	745	1698 (1660, 1778)	98	1738 (1585, 1950)	0.824	75	1905 (1698, 2138)	0.139
	28-32 weeks	730	1660 (1585, 1698)	92	1698 (1549, 1862)	0.868	70	2042 (1820, 2291)	0.001
	≥34 weeks	659	2570 (2512, 2692)	82	2818 (2570, 3162)	0.201	57	3311 (2951, 3802)	0.000
PlGF (pg/ml)	11-14 weeks	746	37 (36, 38)	98	35 (32, 38)	0.243	75	35 (32, 39)	0.593
	18-22 weeks	745	269 (257, 275)	98	245 (224, 269)	0.261	75	245 (219, 275)	0.364
	28-32 weeks	730	617 (589, 646)	92	575 (501, 676)	0.718	70	537 (457, 631)	0.270
	≥34 weeks	659	380 (355, 398)	82	324 (269, 380)	0.178	57	339 (275, 427)	0.585
sFlt-1/ PlGF ratio	11-14 weeks	746	42.7 (40.7, 44.7)	98	50.1 (44.7, 55.0)	0.013	75	50.1 (43.7, 56.2)	0.042
	18-22 weeks	745	6.5 (6.2, 6.8)	98	7.1 (6.3, 7.9)	0.201	75	7.8 (6.8, 8.9)	0.027
	28-32 weeks	730	2.7 (2.5, 2.9)	92	2.9 (2.5, 3.5)	0.643	70	3.8 (3.1, 4.6)	0.005
	≥34 weeks	659	6.9 (6.3, 7.4)	82	8.9 (6.9, 11.2)	0.100	57	9.8 (7.4, 13.2)	0.044

BP, blood pressure; CI, confidence interval.

*Means (95%CI) are adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes; presented as geometric means.

†statistically significant difference between normal BP and elevated BP groups.

‡statistically significant difference between normal BP and hypertension group.

Higher levels of DBP and MAP in early pregnancy were significantly associated with higher log-transformed sFlt-1 values throughout pregnancy. Meanwhile, higher SBP levels were significantly associated with lower log-transformed PlGF levels both at 18-22 weeks (β= -0.02 per 10 mmHg SBP, *P*=0.011) and at 28-32 weeks (β= -0.02 per 10 mmHg SBP, *P*=0.031). Thus, rises in maternal SBP, DBP and MAP in the first trimester were significantly associated with higher sFlt-1/PlGF ratios during pregnancy (supplementary table 1).

Table 4 presents the significant association between blood pressure in early pregnancy and risks of preeclampsia (OR 2.5, 95% CI 1.5-4.0 per 10 mmHg SBP; OR 4.3, 95% CI 2.3-7.9 per 10 mmHg DBP; OR 4.1, 95% CI 2.2-7.7 per 10 mmHg MAP, respectively) after adjustment of potential confounders. Preterm preeclampsia was more closely associated with higher DBP than SBP (OR 6.0, 95% CI 2.3-7.9 per 10 mmHg DBP *vs.* OR 1.9, 95% CI 0.9-3.8 per 10 mmHg SBP).

Table 4. Logistic regression analysis for maternal blood pressure at 11-14weeks and adverse pregnancy outcomes.

Variable	Preeclampsia		Preterm preeclampsia		Term preeclampsia	
	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)
SBP (10mmHg)	3.0 (1.9, 4.6) †	2.5 (1.5, 4.0) †	1.9 (1.1, 3.5)	1.9 (0.9, 3.8)	4.1 (2.2, 7.8) †	3.2 (1.6, 6.4) †
DBP (10mmHg)	5.2 (2.9, 9.3) †	4.3 (2.3, 7.9) †	5.8 (2.4, 14.4) †	6.0 (2.3, 16.2) †	4.4 (2.1, 9.1) †	3.1 (1.4, 6.8) †
MAP (10mmHg)	5.1 (2.8, 9.0) †	4.1 (2.2, 7.7) †	5.1 (2.1, 11.9) †	5.6 (2.0, 15.5) †	4.7 (2.2, 9.7) †	3.3 (1.5, 7.4) †

OR, odds ratio; CI, confidence interval; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure

*Adjusted for maternal age, race, education, maternal body mass index at 11-14 weeks gestation and diabetes mellitus.

†$P < 0.01$.

Discussion

Our study confirmed that higher early pregnancy blood pressure levels were prospectively associated with increased risks of preeclampsia, including preterm and term preeclampsia. Furthermore, women with elevated blood pressure in early pregnancy already had a higher sFlt-1 level and sFlt-1/PlGF ratio in early gestation and throughout pregnancy. In contrast, PlGF levels in these women remained normal throughout gestation.

It is well established that women with chronic hypertension have several times the risk of preeclampsia than normotensive women.[14] However, the pathogensis is poorly understood and what role these angiogenic and anti-angiogenic factors play remains unclear. Although syncytiotrophoblast is a major source of sFlt-1 production, peripheral blood monocytes produce a small amount of sFlt-1 further stimulated by inflammation.[15,16] As chronic hypertension is often related to a chronic inflammatory status,[17] the slightly increased sFlt-1 level in hypertensive women in early pregnancy, as observed in our study, may reflect the chronic inflammatory status in early pregnancy. Our results showed that hypertensive women in early pregnancy might have an imbalanced angiogenic factors levels and such imbalanced angiogenic environment tended to continue during pregnancy, which might be associated with the increased risks of preeclampsia.

On the other hand, maternal circulating PlGF is highly expressed by the placenta

during pregnancy. It has both vasculogenic and angiogenic functions [18] and its level is likely to reflect the placental health conditions. For example, low PlGF concentrations preceding clinical onset of preeclampsia often occur in early-onset rather than late-onset preeclampsia.[19] Women who develop preeclampsia with fetal growth restriction (FGR) have further decreased PlGF levels compared with women who develop preeclampsia without FGR.[20-22] Early-onset preeclampsia and placenta-derived FGR are associated with placenta pathology such as incomplete remodeling of spiral arteries, acute atherosis and thrombosis in spiral arteries and syncytiotrophoblast necrosis.[23,24] In our study, women with elevated blood pressure in early pregnancy, the PlGF level remained by and large normal throughout pregnancy and newborn's birth weight was not significantly different among the three groups. Thus, our findings seem to suggest that the placental implantation and development might not be impaired in these women.

As poor placentation is not a unique cause of developing preeclampsia, enhanced placental oxidative and endoplasmic reticulum stress and increased maternal systemic inflammatory responses are thought to play crucial roles in preeclampsia as well.[25,26] Thus, preexisting endothelial dysfunction in hypertensive women could be exacerbated as a result of physiological burden of pregnancy even without an abnormal placentation.[27] Taking all things considered, we propose that the imbalanced angiogenic factors environment and, perhaps more importantly, preexisting endothelial susceptibility and dysfunction, may play a critical role in the development

of preeclampsia in women with elevated blood pressure in early pregnancy.

To our best knowledge, this was the first prospective cohort study that illustrated the dynamic changes of angiogenic and anti-angiogenic factors throughout pregnancy in women with different blood pressure status in early pregnancy. The NORA cohort was a well-performed prospective study with comprehensive information including clinical, biophysical and biochemical markers. The follow-up rate was 99.1% (926/934) at the end of pregnancy. Measurements of blood pressure and serum angiogenic factors were performed according to standardized protocols. In our study, three women reported using antihypertensive medications during pregnancy; thus, the results should not be affected by the medication issue. On the other hand, as most of our participants were low-risk pregnant women, our results may not be applicable to high-risk women. As it was an observational study, the potential residual confounding and selection bias might have some impacts on our results.

Conclusion

Women with elevated blood pressure in early pregnancy already had a higher sFlt-1/PlGF ratio in early gestation and throughout pregnancy, and an increased risk of preeclampsia. In contrast, PlGF levels in these women remained normal throughout gestation. Our findings suggest that the imbalanced angiogenic factors levels throughout gestation might play a crucial role in developing preeclampsia in women with preexisting elevated blood pressure. Our study also supports that preconception

or early pregnancy high blood pressure, defined as SBP≥130 mmHg or DBP≥80 mmHg according to 2017 ACC/AHA guideline, should cause clinical awareness both during pregnancy and in their later life.

Acknowledgments

We thank all the NORA cohort (Neonatal and Obstetrics Risks Assessment) participants for their commitment to the study, the NORA cohort investigators and staff.

Author Contributions

JZ: performed the statistical analysis, searched literature and drafted the manuscript. JZ (corresponding author): had the original idea, provided guidance for the statistical analysis and revised the manuscript. MJN, BC, GSY and KHT: participated in the data collection, reviewed and revised the manuscript.

Funding: The authors received no finical support for the research, authorship, and/or publication of this article.

Competing interests: None declared.

Patient consent for publication: Obtained.

Ethics approval: This study was approved by the SingHealth Centralised Institutional Review Board Ethics Committee, Singapore (CIRB Ref No. 2010/214/D).

Provenance and peer review: Not commissioned; externally peer reviewed.

Data availability statement: All data relevant to the study are included in the article or uploaded as supplementary information.

References

1. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A* 1993; 90:10705-10709.
2. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, *et al*. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; 350(7): 672-83.
3. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, *et al.* Predictive Value of the sFlt-1: PlGF Ratio in Women with Suspected Preeclampsia. N E*ngl J Med* 2016 Jan 7;374(1):13-22.
4. NICE. PlGF-based Testing to Help Diagnose Suspected Pre-eclampsia (Triage PlGF Test, Elecsys Immunoassay sFlt-1/PlGF Ratio, DELFIA Xpress PlGF 1-2-3 Test, and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio). Diagnostics Guidance 23. London: NICE, 2016.
5. 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. *J Clin Invest* 2003;111(5):649-58.
6. Sela S, Itin A, Natanson-Yaron S, Greenfield C, Goldman-Wohl D, Yagel S, *et al.* A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-type-specific splicing and implications to vascular endothelial growth factor homeostasis and preeclampsia. *Circ Res* 2008;102(12):1566-74.
7. Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem* 1997;272(38):23659-67.
8. Warrington JP, George EM, Palei AC, Spradley FT, Granger JP. Recent advances in the understanding of the pathophysiology of preeclampsia. *Hypertension* 2013;62(4):666-73.
9. Yang J, Pearl M, DeLorenze GN, Romero R, Dong Z, Jelliffe-Pawlowski L, *et al.*

Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. *Am J Obstet Gynecol.* 2016; 215(3): 359.e1-9.

10. Ng QJ, Zhang J, Dai F, Ng MJ, Razali NS, Win NM, *et al.* Neonatal and Obstetric Risk Assessment (NORA) Pregnancy Cohort Study in Singapore. *Int J Gynaecol Obstet* 2018; 4(1): 31-37.
11. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, *et al.* Recommendations for blood pressure measurement in humans and experimental animals. *Circulation* 2005; 111:697-716.
12. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; 20(1): IX-XIV.
13. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, *et al.* 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018;71(6):1269-1324.
14. Bartsch E, Medcalf KE, Park AL, Ray JG; High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. *BMJ* 2016;353: i1753.
15. Rajakumar A, Michael HM, Rajakumar PA, Shibata E, Hubel CA, Karumanchi SA, *et al.* Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. *Placenta* 2005;26(7):563-73.
16. Freeman DJ, McManus F, Brown EA, Cherry L, Norrie J, Ramsay JE, *et al.* Short- and long-term changes in plasma inflammatory markers associated with

preeclampsia. *Hypertension* 2004;44(5):708-14.

17. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *Biomed Res Int* 2014; 2014: 406960.
18. De Falco S. The discovery of placenta growth factor and its biological activity. *Exp Mol Med* 2012;44(1):1-9.
19. McElrath TF, Lim KH, Pare E, Rich-Edwards J, Pucci D, Troisi R, *et al.* Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. *Am J Obstet Gynecol* 2012;207(5): 407.e1-7.
20. Powers RW, Roberts JM, Plymire DA, Pucci D, Datwyler SA, Laird DM, *et al.* Low placental growth factor across pregnancy identifies a subset of women with preterm preeclampsia: type 1 versus type 2 preeclampsia? *Hypertension* 2012;60(1):239-46.
21. 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. *J Matern Fetal Neonatal Med* 2008;21(1):9-23.
22. Chaiworapongsa T, Romero R, Whitten AE, Korzeniewski SJ, Chaemsaithong P, Hernandez-Andrade E, *et al.* The use of angiogenic biomarkers in maternal blood to identify which SGA fetuses will require a preterm delivery and mothers who will develop pre-eclampsia. *J Matern Fetal Neonatal Med* 2016;29(8):1214-28.
23. Nelson DB, Ziadie MS, McIntire DD, Rogers BB, Leveno KJ. Placental pathology suggesting that preeclampsia is more than one disease. *Am J Obstet Gynecol* 2014;210(1): 66.e1-7.
24. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. *Am J Obstet Gyneco.* 2018;218(2S): S745-S761.
25. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet*

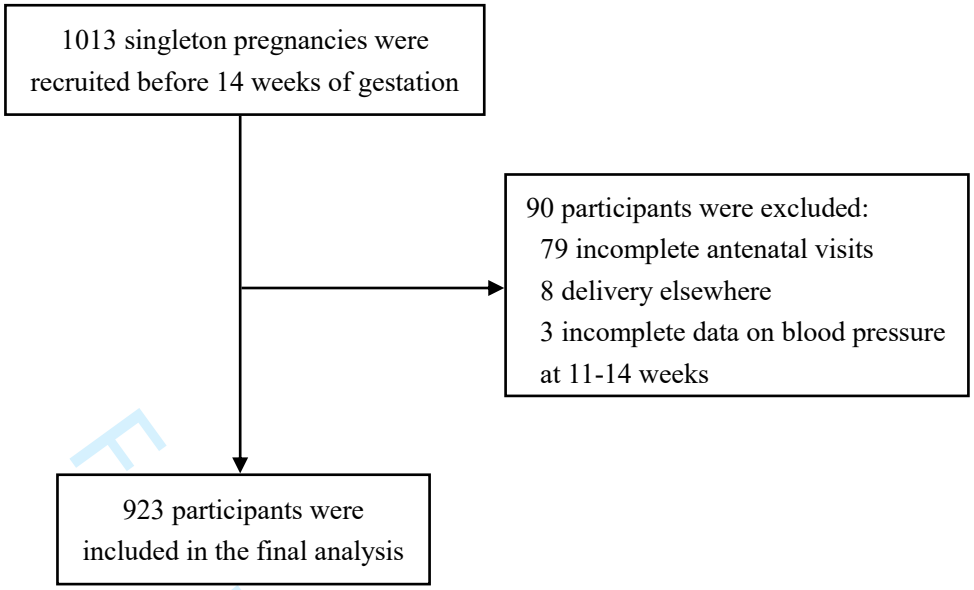
2010;376(9741):631-44.

26. Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? *Placenta* 2014;35 Suppl: S20-5.

27. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am J Obstet Gynecol* 1996;175(5):1365-70.

Figure legends

Figure 1. Flowchart of participants.



Supplementary table 1. Associations of maternal blood pressure at 11-14weeks with serum angiogenic factors levels during pregnancy.

Variable	Time points	Log sFlt-1		Log PlGF		Log sFlt-1/ PlGF ratio	
		β (95% CI) *	*P* value	β (95% CI) *	*P* value	β (95% CI) *	*P* value
Systolic blood pressure	11-14 weeks	0.01 (0.004, 0.03)	0.009	-0.01 (-0.02, 0.0004)	0.057	0.03 (0.01, 0.04)	< 0.001
(10mmHg)	18-22 weeks	0.01 (-0.01, 0.02)	0.227	-0.02 (-0.03, -0.004)	0.011	0.02 (0.01, 0.04)	0.003
	28-32 weeks	0.01 (-0.001, 0.02)	0.068	-0.02 (-0.04, -0.002)	0.031	0.03 (0.01, 0.06)	0.007
	⩾34 weeks	0.02 (0.01, 0.03)	0.009	-0.02 (-0.05, 0.001)	0.059	0.04 (0.01, 0.07)	0.011
Diastolic blood pressure	11-14 weeks	0.02 (0.01, 0.04)	0.002	-0.01 (-0.03, 0.001)	0.069	0.04 (0.02, 0.05)	< 0.001
(10mmHg)	18-22 weeks	0.02 (0.004, 0.04)	0.014	-0.01 (-0.02, 0.01)	0.388	0.03 (0.01, 0.05)	0.008
	28-32 weeks	0.04 (0.02, 0.05)	< 0.001	-0.02 (-0.04, 0.004)	0.104	0.05 (0.03, 0.08)	< 0.001
	⩾34 weeks	0.04 (0.02, 0.06)	< 0.001	-0.02 (-0.05, 0.01)	0.134	0.06 (0.02, 0.10)	0.003
Mean arterial pressure	11-14 weeks	0.02 (0.01, 0.04)	0.002	-0.02 (-0.03, -0.001)	0.043	0.04 (0.02, 0.06)	< 0.001
(10mmHg)	18-22 weeks	0.02 (0.001, 0.03)	0.044	-0.01 (-0.02, 0.01)	0.506	0.02 (0.002, 0.04)	0.030
	28-32 weeks	0.03 (0.01, 0.04)	0.001	-0.02 (-0.05, 0.001)	0.058	0.05 (0.02, 0.08)	0.001
	⩾34 weeks	0.04 (0.02, 0.05)	< 0.001	-0.03 (-0.06, -0.003)	0.029	0.07 (0.03, 0.11)	< 0.001

Abbreviations: CI, confidence interval.

*Adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test.

Supplementary table 2. Maternal serum angiogenic factors levels during pregnancy by preeclampsia in women with different BP status in early pregnancy.

Angiogenic factors	Time points	Normal BP			Elevated BP			Hypertension (stage 1 and stage 2)		
		PE (n=6)	Non-PE (n=744)	*P* value	PE (n=3)	Non-PE (n=95)	*P* value	PE (n=12)	Non-PE (n=63)	*P* value
		mean (95%CI) *	mean (95%CI) *		mean (95%CI) *	mean (95%CI) *		mean (95%CI) *	mean (95%CI) *	
sFlt-1 (pg/ml)	11-14 weeks	1514 (1096, 2089)	1622 (1549, 1660)	0.718	1778 (1096, 2884)	1622 (1514, 1778)	0.756	1349 (1122, 1622)	1660 (1549, 1820)	0.048
	18-22 weeks	1698 (1175, 2512)	1738 (1660, 1778)	0.925	1479 (813, 2692)	1660 (1514, 1862)	0.692	1738 (1288, 2291)	1738 (1514, 1995)	0.960
	28-32 weeks	3090 (2188, 4365)	1698 (1622, 1738)	<0.001	1995 (1148, 3467)	1549 (1413, 1698)	0.388	3020 (2188, 4074)	1622 (1445, 1862)	<0.001
	⩾34 weeks	6026 (3548, 10233)	2630 (2512, 2692)	0.002	3715 (2042, 6607)	2692 (2399, 2951)	0.295	6026 (3802, 9550)	2951 (2570, 3388)	0.005
PlGF (pg/ml)	11-14 weeks	29.5 (21.4, 41.7)	37.2 (36.3, 38.0)	0.199	32.4 (20.0, 53.7)	36.3 (33.1, 38.9)	0.716	24.6 (19.1, 30.9)	35.5 (32.4, 39.8)	0.005
	18-22 weeks	208.9 (144.5, 309.0)	269.2 (263.0, 281.8)	0.191	182.0 (114.8, 288.4)	239.9 (218.8, 257.0)	0.257	151.4 (120.2, 190.6)	234.4 (213.8, 263.0)	0.001
	28-32 weeks	269.2 (158.5, 457.1)	631.0 (602.6, 660.7)	0.002	323.6 (166.0, 645.7)	562.3 (501.2, 645.7)	0.118	182.0 (120.2, 269.2)	588.8 (489.8, 691.8)	<0.001
	⩾34 weeks	169.8 (67.6, 426.6)	380.2 (363.1, 407.4)	0.083	208.9 (85.1, 512.9)	316.2 (269.2, 371.5)	0.372	147.9 (66.1, 323.6)	323.6 (257.0, 416.9)	0.059
sFlt-1/ PlGF ratio	11-14 weeks	50.1 (33.1, 77.6)	42.7 (41.7, 44.7)	0.454	53.7 (28.8, 100.0)	45.7 (40.7, 50.1)	0.591	56.2 (42.7, 74.1)	46.8 (41.7, 52.5)	0.235

18-22 weeks	8.1 (5.0, 12.9)	6.5 (6.2, 6.6)	0.323	8.1 (4.2, 15.9)	7.1 (6.3, 7.9)	0.674	11.2 (7.9, 16.2)	7.4 (6.3, 8.5)	0.029
28-32 weeks	11.5 (6.0, 21.9)	2.7 (2.5, 2.8)	<0.001	6.0 (2.4, 15.1)	2.8 (2.4, 3.2)	0.093	16.6 (9.8, 28.2)	2.8 (2.2, 3.6)	<0.001
⩾34 weeks	35.5 (10.5, 120.2)	6.8 (6.3, 7.4)	0.008	17.8 (4.9, 64.6)	8.5 (6.8, 10.7)	0.269	40.7 (14.5, 114.8)	9.1 (6.6, 12.6)	0.008

BP, blood pressure; CI, confidence interval; PE, preeclampsia.

*Means (95%CI) are adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes; presented as geometric means.

Supplementary table 3. Logarithm-transformed maternal serum angiogenic factors levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Angiogenic factors	Time points	Normal BP		Elevated BP				Hypertension (stage 1 and stage 2)			
		N	mean (95%CI) *	N	mean (95%CI) *	mean difference (95%CI) *†	*P* value †	N	mean (95%CI) *	mean difference (95%CI) *‡	*P* value ‡
sFlt-1 (pg/ml)	11-14 weeks	746	3.20 (3.19, 3.21)	98	3.24 (3.20, 3.27)	0.04 (-0.01, 0.08)	0.096	75	3.24 (3.20, 3.29)	0.05 (-0.01, 0.10)	0.079
	18-22 weeks	745	3.23 (3.22, 3.25)	98	3.24 (3.20, 3.29)	0.01 (-0.04, 0.06)	0.824	75	3.28 (3.23, 3.33)	0.05 (-0.01, 0.11)	0.139
	28-32 weeks	730	3.22 (3.20, 3.23)	92	3.23 (3.19, 3.27)	0.01 (-0.04, 0.06)	0.868	70	3.31 (3.26, 3.36)	0.09 (0.03, 0.15)	0.001
	⩾34 weeks	659	3.41 (3.40, 3.43)	82	3.45 (3.41, 3.50)	0.04 (-0.02, 0.10)	0.201	57	3.52 (3.47, 3.58)	0.11 (0.04, 0.17)	0.000
PlGF (pg/ml)	11-14 weeks	746	1.57 (1.56, 1.58)	98	1.54 (1.50, 1.58)	-0.03 (-0.08, 0.01)	0.243	75	1.55 (1.50, 1.59)	-0.02 (-0.07, 0.03)	0.593
	18-22 weeks	745	2.43 (2.41, 2.44)	98	2.39 (2.35, 2.43)	-0.03 (-0.08, 0.02)	0.261	75	2.39 (2.34, 2.44)	-0.03 (-0.09, 0.03)	0.364
	28-32 weeks	730	2.79 (2.77, 2.81)	92	2.76 (2.70, 2.83)	-0.02 (-0.10, 0.05)	0.718	70	2.73 (2.66, 2.80)	-0.06 (-0.14, 0.03)	0.270
	⩾34 weeks	659	2.58 (2.55, 2.60)	82	2.51 (2.43, 2.58)	-0.07 (-0.17, 0.02)	0.178	57	2.53 (2.44, 2.63)	-0.05 (-0.16, 0.07)	0.585
sFlt-1/ PlGF ratio	11-14 weeks	746	1.63 (1.61, 1.65)	98	1.70 (1.65, 1.74)	0.07 (0.01, 0.13)	0.013	75	1.70 (1.64, 1.75)	0.07 (0.01, 0.13)	0.042
	18-22 weeks	745	0.81 (0.79, 0.83)	98	0.85 (0.80,	0.05 (-0.02, 0.11)	0.201	75	0.89 (0.83,	0.08 (0.01, 0.15)	0.027

				0.90)				0.95)		
28-32 weeks	730	0.43 (0.40, 0.46)	92	0.46 (0.39, 0.54)	0.03 (-0.06, 0.13)	0.643	70	0.58 (0.49, 0.66)	0.15 (0.04, 0.25)	0.005
⩾34 weeks	659	0.84 (0.80, 0.87)	82	0.95 (0.84, 1.05)	0.11 (-0.02, 0.24)	0.100	57	0.99 (0.87, 1.12)	0.16 (0.01, 0.31)	0.044

BP, blood pressure; CI, confidence interval.

*Means (95%CI) are adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes;

†statistically significant difference between normal BP and elevated BP groups.

‡statistically significant difference between normal BP and hypertension group.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 2, line 9
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5, line 14-22
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 6, line 1-4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 6, line 8-9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 6, line 8-22
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 7, line 2-14
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 7, line 16 ~ Page 8, line 12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 8, line 14 ~ Page 9, line 10
Bias	9	Describe any efforts to address potential sources of bias	Page 7, line 4-7
Study size	10	Explain how the study size was arrived at	Page 7, line 7-14
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8, line 6-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 9, line 12~ Page 10, line 10
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 10, line 17-20
		(b) Give reasons for non-participation at each stage	Page 7,

			line 8-9
		(c) Consider use of a flow diagram	Page 7, figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 11, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Page 15, Table 3
		(c) Summarise follow-up time (eg, average and total amount)	Page 15, Table 3
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 15, Table 3
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 15, Table 3, Page 17, Table 4
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 18, line 2-7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 20, line 9-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 20, line 17-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 20, line 11-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 22, Line 12-13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032237.R2
Article Type:	Original research
Date Submitted by the Author:	04-Nov-2019
Complete List of Authors:	Zhu, Jing; MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China Zhang, Jun; Ministry of Education–Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine Ng, Mor Jack; KK Women's and Children's Hospital, Division of Obstetrics and Gynaecology Chern, Bernard; KK Women's and Children's Hospital Yeo, George S. H.; KK Women's and Children's Hospital, Singapore Tan, Kok Hian; KK Women's and Children's Hospital, Division of Obstetrics and Gyneacology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Epidemiology
Keywords:	blood pressure, soluble fms-like tyrosine kinase 1, placental growth factor, preeclampsia

Angiogenic Factors during Pregnancy in Asian Women with Elevated Blood Pressure in Early Pregnancy and the Risk of Preeclampsia: a longitudinal cohort study

Jing Zhu,[1] Jun Zhang,[1,2] Mor Jack Ng,[2] Bernard Chern,[2] George SH Yeo,[3] Kok Hian Tan [2]

[1]MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[2]Division of Obstetrics and Gynaecology, KK Women's and Children's Hospital, Singapore

[3]Department of Maternal and Fetal Medicine, KK Women's and Children's Hospital, Singapore

Corresponding Author: Dr. Jun Zhang

Xinhua Hospital, Shanghai Jiao Tong University School of Medicine

No. 1665 Kongjiang Road, Shanghai, 200092, China

Telephone: 86-21-25078871

E-mail: junjimzhang@sina.com; zhangjun@xinhuamed.com.cn

Word count: abstract 262; main text 2525

Number of tables: 4

Number of figures: 1

Abstract

Objective

It remains unclear what roles placenta-originated angiogenic factors play in the pathogenesis of preeclampsia among hypertensive women. We compared maternal soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) levels throughout pregnancy in women with normal blood pressure (BP), elevated BP and hypertension in early pregnancy and their risks of developing preeclampsia.

Design

A prospective cohort study.

Setting

KK Women's and Children's Hospital, Singapore.

Participants

923 women with singleton pregnancy less than 14 weeks of gestation were included in the prospective Neonatal and Obstetrics Risks Assessment cohort between September 2010 and October 2014. Systolic, diastolic, mean arterial blood pressure (MAP) were measured at 11-14 weeks.

Primary and secondary outcomes

Maternal serum sFlt-1, PlGF and sFlt-1/PlGF ratio were tested at 11-14 weeks, 18-22 weeks, 28-32 weeks and 34 weeks onwards of gestation. Preeclampsia was main pregnancy outcome.

Results

Women were divided based on their BP in early pregnancy: normal (N=750), elevated BP (N=98) and hypertension (N=75). Maternal sFlt-1 levels and sFlt-1/PlGF ratios

were higher in hypertensive women throughout pregnancy, but maternal PlGF levels were not significantly lower. Rise in maternal systolic, diastolic BP and MAP at 11-14 weeks were significantly associated with higher sFlt-1/PlGF ratios during pregnancy. A 10mmHg increase in MAP was associated with a 5.6-fold increase in risk of preterm preeclampsia and a 3.3-fold increase in risk of term preeclampsia, respectively.

Conclusion

Women with elevated blood pressure in early pregnancy already had a higher sFlt-1/PlGF ratio in early gestation and throughout pregnancy, and an increased risk of preeclampsia. In contrast, PlGF levels in these women remained normal.

Key words

blood pressure, soluble fms-like tyrosine kinase 1, placental growth factor, preeclampsia

Strengths and limitations of the study

- This study was based on a well-performed prospective cohort with comprehensive information on clinical, biophysical and biochemical markers.
- Covariance analysis was performed to compare differences of angiogenic factors values among groups; multivariable logistic regression analysis was performed to evaluate the association between early pregnancy blood pressure and pregnancy outcomes.
- Given that most of our participants were low-risk pregnant women, our results may not be applicable to high-risk women.

Introduction

The imbalance in placenta-originated angiogenic factors has been found to play an important role in the pathogenesis of preeclampsia in recent years. Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) are the most studied proteins. sFlt-1, a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1, is a circulating anti-angiogenic protein that inhibits proangiogenic factors-VEGF and placental growth factor (PlGF) signaling in the vasculature.[1] High levels of circulating sFlt-1 and low levels of PlGF were observed in women with established preeclampsia and even before the onset of clinical symptoms. [2,3] These promising findings have been adopted and recommended by the National Institute for Clinical Excellence (NICE) to rule out preeclampsia in women presenting with clinical suspicion.[4]

Numerous studies focused on the pathological effects of excess sFlt-1 on endothelial function. It was observed that overexpression of sFlt-1 produced a preeclampsia-like syndrome of hypertension, proteinuria and glomerular endotheliosis in experimental animals.[5] sFlt-1 is largely produced by syncytiotrophoblast and secreted into maternal circulation.[6] Placental hypoxia may be one of the main triggers of inducing abundant sFlt-1 expression and leading to hypertensive complications.[7,8] However, this hypothesis may not totally explain why women with elevated blood pressure before pregnancy have a substantially higher risk of preeclampsia. Besides, evidence suggests that there might be some racial differences in maternal angiogenic and anti-

angiogenic factors. [9] Thus, the objective of this study was to examine the dynamic changes of angiogenic and anti-angiogenic factors throughout pregnancy in Asian women with elevated blood pressure in early gestation and their risks of developing preeclampsia later in pregnancy.

Methods

Study design

The Neonatal and Obstetrics Risks Assessment (NORA) study was a prospective cohort conducted at the KK Women's and Children's Hospital (KKH) in Singapore.[10] The cohort was set up to screen factors associated with adverse perinatal outcomes, with a focus on using clinical, biochemical and biophysical markers to predict the risks of pregnancy complications in early pregnancy. In brief, detailed interviews, ultrasound scans and blood sample collections were performed at recruitment (11 to 14 weeks), 18 to 22 weeks, 28 to 32 weeks and 34 weeks onward, respectively. All 4 antenatal visits also included measurement of maternal height and weight; recording of blood pressure by validated automated devices according to the recommendations of the American Heart Association.[11] Participants were closely followed up till their postnatal discharge from the hospital. Information on pregnancy complications, labor and delivery and neonatal outcomes was collected through medical chart review. The study protocol was approved by the SingHealth Centralised Institutional Review Board Ethics Committee, Singapore (CIRB Ref No. 2010/214/D), and a written informed consent was obtained from all participating women.

Study population

The NORA cohort recruited women with confirmed singleton pregnancies less than 14 weeks of gestation between September 2010 and October 2014. The exclusion criteria were multiple gestation, severe medical conditions such as chronic renal disease or systemic lupus erythematosus and pregnancies complicated by aneuploidy or fetal anomaly. Gestational age was confirmed by ultrasound at recruitment. A total of 1013 women were enrolled and 934 of them completed all 4 antenatal visits. 8 participants were delivered elsewhere, leaving 926 eligible women in the cohort. To evaluate the impacts of maternal blood pressure in early pregnancy on angiogenic factors levels and pregnancy outcomes, we used blood pressure at recruitment (11-14 weeks) to classify women into normal, elevated and hypertension groups. As 3 women did not have blood pressure records at recruitment (11-14 weeks), we included 923 participants for the final analysis (Figure 1).

Diagnosis

Preeclampsia was defined according to the guidelines of International Society for the Study of Hypertension in Pregnancy [12]: systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least two occasions four hours apart after 20 weeks of gestation in a previously normotensive women, and proteinuria: urinary albumin ≥300mg/24 hours urine collection or ≥1+ dipstick. We used the gestational age at delivery to divide cases of preeclampsia into term (≥37

weeks) and preterm term (< 37 weeks). Gestational hypertension was defined as newly onset hypertension after 20 weeks of gestation without proteinuria. Chronic hypertension was defined as women with history of hypertension before conception or the presence of hypertension before 20 weeks of gestation.

To explore the relationship between maternal blood pressure levels in the first trimester and pregnancy outcome, we followed the 2017 guideline from the American College of Cardiology and the American Heart Association (ACC/AHA).[13] Normal blood pressure was defined as SBP < 120 mmHg and DBP < 80 mmHg; elevated blood pressure was defined as SBP 120-129 mmHg and DBP < 80 mmHg; hypertension stage 1 as SBP 130-139 mmHg or DBP 80-89 mmHg and hypertension stage 2 as SBP≥140 mmHg or DBP ≥90 mmHg.

Measurement of blood pressure

Blood pressure was taken by validated automated devices which were calibrated periodically. The women were in the seated position and their arms were supported. A correct cuff size was used and the middle of cuff was positioned on woman's upper arm at the level of the right atrium. After a five-minute rest, blood pressure was measured by trained nurses and three recordings were made at 1-minute intervals. We calculated SBP and DBP as the average of the three measurements. Mean arterial pressure (MAP) was calculated from SBP and DBP measures using the following formula: MAP=DBP+1/3×(SBP-DBP). Blood pressure was modeled continuously in

units of 10 mm of mercury (mmHg).

Measurements of angiogenic factors

About 8 ml of maternal venous blood was collected in non-heparinised tubes at each antenatal visit. It was then centrifuged at 2000rpm for 15 minutes before separating and storing serum samples at -80°C for subsequent analysis. Serum levels of sFlt-1 and PlGF were determined by means of the fully automated Elecsys assays on an electrochemiluminescence immunoassay platform (cobas e411 analyzers, Roche Diagnostics). The detection limit was approximately 6 pg/ml for sFlt-1 and <2 pg/ml for PlGF.

Statistical analysis

Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. Numeric data were expressed as mean (standard deviation) or as median (interquartile range) for normally and non-normally distributed data, respectively. Maternal characteristics, pregnancy outcomes and maternal blood pressure levels were compared among normal, elevated blood pressure and hypertension (stage 1 and stage 2) groups using one-way ANOVA or Kruskal-Wallis tests for continuous variables and χ^2 analysis for categorical variables. Covariance analysis and Dunnett test was used to compare differences of logarithm-transformed angiogenic factors values among groups adjusted for covariants. Geometric means and 95% confidence intervals were calculated by taking the exponent of the logarithm transformed mean.

Linear regression analysis was performed to assess the association of blood pressure (10 mmHg) at recruitment with logarithm-transformed angiogenic factors values during pregnancy. Covariance analysis and linear regression models were adjusted for maternal race, smoking during pregnancy, body mass index (BMI) and gestational age at blood collection as covariant. Logistic regression analysis was performed to evaluate the association between early pregnancy blood pressure (10 mmHg) and pregnancy outcomes with adjustment of potential confounders, including maternal age, race, education, maternal BMI at recruitment, chronic hypertension and preexisting diabetes mellitus. We used SAS version 9.4 (Cary, NC) for all statistical analyses.

Patient and public involvement

Patients and the public were not directly involved in the design, conduct or reporting in our study.

Results

A total of 923 participants in the NORA cohort were included in this analysis. Based on maternal blood pressure at recruitment at 11-14 weeks of gestation, 750 women were classified as normal blood pressure; 98 women as elevated blood pressure, and 75 women as hypertension (stage 1 and stage 2). A comparison of maternal characteristics and pregnancy outcomes are given in Table 1. Women with hypertension (stage 1 and stage 2) in the first trimester were slightly older than the

other two groups. Chinese women contributed a greater proportion of hypertension (49.3%) than Indian (8.0%) and Malay (30.7%). A higher incidence of maternal overweight (BMI ≥25 kg/m^2) and obesity (BMI ≥30 kg/m^2) was observed in the elevated blood pressure group and hypertension group than in the normal blood pressure group.

Table 1. Characteristics and pregnancy outcomes by maternal blood pressure at 11-14weeks in the NORA cohort.

Variables	Normal BP (n=750)	Elevated BP (n=98)	Hypertension (stage 1 and stage2) (n=75)	*P* value
Maternal age (year), median (IQR)	30.0 (26.0-34.0)	30.0 (26.0-35.0)	32.0 (29.0-35.0)	< 0.001
Race, n (%)				0.012
Chinese	393 (52.3)	38 (38.8)	37 (49.3)	
Indian	88 (11.7)	6 (6.1)	6 (8.0)	
Malay	186 (24.8)	41 (41.8)	23 (30.7)	
Others	84 (11.2)	13 (13.3)	9 (12.0)	
Parity, n (%)				0.091
0	419 (55.9)	49 (50.0)	33 (44.0)	
1	236 (31.5)	32 (32.7)	25 (33.3)	
≥ 2	95 (12.7)	17 (17.3)	17 (22.7)	
Maternal education levels, n (%)				0.044
Less than high school	166 (22.2)	28 (28.6)	25 (33.3)	
High school	299 (40.0)	44 (44.9)	23 (30.7)	
College and above	282 (37.8)	26 (26.5)	27 (36.0)	
Married, n (%)	699 (93.2)	90 (91.8)	72 (96.0)	0.544
Smoking during pregnancy, n (%)	19 (2.5)	4 (4.1)	0 (0)	0.230
Maternal BMI at 11-14 weeks of gestation (kg/m2), n (%)				< 0.001
< 18.5	62 (8.3)	2 (2.0)	0 (0)	
18.5-24.9	475 (63.5)	32 (32.7)	16 (21.3)	
25.0-29.9	162 (21.7)	35 (35.7)	32 (42.7)	
≥ 30.0	49 (6.6)	29 (29.6)	27 (36.0)	

Diabetes mellitus, n (%)	7 (0.9)	2 (2.0)	5 (6.7)	< 0.001
ART conception, n (%)	31 (4.1)	4 (4.1)	2 (2.7)	0.826
Antihypertensive medication, n (%)	0 (0)	0 (0)	3 (4.0)	< 0.001
Pregnancy outcomes				
Delivery age (weeks), median (IQR)	39.0 (38.1-39.7)	38.7 (38.0-39.6)	38.3 (37.6-39.1)	0.001
Birth weight (kg), median (IQR)	3.1 (2.9-3.4)	3.1 (2.9-3.5)	3.1 (2.8-3.4)	0.324
Gestational hypertension, n (%)	10 (1.3)	3 (3.1)	5 (6.7)	0.004
Preeclampsia, n (%)	6 (0.8)	3 (3.1)	12 (16.0)	< 0.001
Preterm preeclampsia, n (%)	4 (0.5)	0 (0)	5 (6.7)	< 0.001
Term preeclampsia, n (%)	2 (0.3)	3 (3.1)	7 (9.3)	< 0.001

IQR: interquartile range; BMI: body mass index; ART: assisted reproductive technology; BP: blood pressure.

The prevalence of maternal preconception diabetes was 6.7% in hypertension groups which was significantly higher than that of the other two groups. However, the prevalence of conception with assisted reproductive technology (ART) were not significantly different among the three groups. As expected, the incidence of gestational hypertension (6.7%), preeclampsia (16.0%), preterm preeclampsia (6.7%) and term preeclampsia (9.3%) were the highest in the hypertension group. Women with hypertension had sustainable higher blood pressure levels during pregnancy (Table 2).

Table 2. Maternal blood pressure levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Variables	Normal BP (n=750)	Elevated BP (n=98)	Hypertension (stage 1 and stage2) (n=75)	*P* value
SBP (mmHg), mean±SD				

11-14 weeks	104.9±8.3	123.2±2.6	126.8±11.1	< 0.001
18-22 weeks	105.9±10.1	118.6±9.9	124.0±10.5	< 0.001
28-32 weeks	108.2±10.0	118.1±9.7	123.8±11.4	< 0.001
≥34 weeks	110.5±10.7	119.5±10.6	126.1±15.5	< 0.001
DBP (mmHg), mean±SD				
11-14 weeks	63.6±6.8	71.4±4.4	80.8±7.0	< 0.001
18-22 weeks	62.9±7.0	69.0±7.2	76.3±8.1	< 0.001
28-32 weeks	64.0±6.9	69.8±6.6	76.6±9.0	< 0.001
≥34 weeks	67.4±7.8	73.3±7.3	80.0±11.4	< 0.001
MAP (mmHg), mean±SD				
11-14 weeks	77.3±6.5	88.7±3.2	96.1±6.5	< 0.001
18-22 weeks	77.2±7.2	85.5±7.3	92.2±8.2	< 0.001
28-32 weeks	78.8±7.1	85.9±6.3	92.4±9.1	< 0.001
≥34 weeks	81.8±8.1	88.7±7.6	95.4±11.9	< 0.001

BP: blood pressure; SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure

Table 3 shows the values of angiogenic factors at 4 time points in the NORA participants. Logarithm-transformed serum angiogenic factors levels at 4 time points are presented as well (supplementary table 1). We used covariance analysis to control for potential confounders that were reported to have impact on serum angiogenic proteins levels, including maternal race, smoking, maternal BMI and gestational age at blood collection. Overall, serum sFlt-1 concentrations continued rising throughout

pregnancy. PlGF levels increased from the first trimester, peaked at 28-32 weeks and declined afterwards. Consequently, high levels of sFlt-1/PlGF ratio were observed both at 11-14 weeks and 34 weeks onwards. The dynamic change of serum angiogenic factors during pregnancy was observed in all 3 groups. Maternal serum sFlt-1 and PlGF levels were not significantly different between elevated BP group and normal BP group at 4 time points during pregnancy. In hypertension group, a trend of higher maternal sFlt-1 concentrations was observed from early pregnancy and it was dramatically increased during the third trimester compared with sFlt-1 levels in normotensive women. In contrast, PlGF concentrations were not significantly different between hypertension group and normal BP group. Thus, sFlt-1/PlGF ratio in hypertensive women was significantly higher throughout pregnancy than that in normotensive women. Comparisons of angiogenic factors levels throughout gestation in women with or without preeclampsia in each group are presented in supplementary table 2.

Table 3. Maternal serum angiogenic factors levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Angiogenic factors	Time points	Normal BP		Elevated BP			Hypertension (stage 1 and stage 2)		
		N	mean (95%CI) *	N	mean (95%CI) *	*P* value †	N	mean (95%CI) *	*P* value ‡
sFlt-1 (pg/ml)	11-14 weeks	746	1585 (1549, 1622)	98	1722 (1585, 1862)	0.096	75	1758 (1585, 1950)	0.079
	18-22 weeks	745	1698 (1660, 1778)	98	1738 (1585, 1950)	0.824	75	1905 (1698, 2138)	0.139
	28-32 weeks	730	1660 (1585, 1698)	92	1698 (1549, 1862)	0.868	70	2042 (1820, 2291)	0.001
	≥34 weeks	659	2570 (2512, 2692)	82	2818 (2570, 3162)	0.201	57	3311 (2951, 3802)	0.000
PlGF (pg/ml)	11-14 weeks	746	37 (36, 38)	98	35 (32, 38)	0.243	75	35 (32, 39)	0.593
	18-22 weeks	745	269 (257, 275)	98	245 (224, 269)	0.261	75	245 (219, 275)	0.364
	28-32 weeks	730	617 (589, 646)	92	575 (501, 676)	0.718	70	537 (457, 631)	0.270
	≥34 weeks	659	380 (355, 398)	82	324 (269, 380)	0.178	57	339 (275, 427)	0.585
sFlt-1/ PlGF ratio	11-14 weeks	746	42.7 (40.7, 44.7)	98	50.1 (44.7, 55.0)	0.013	75	50.1 (43.7, 56.2)	0.042
	18-22 weeks	745	6.5 (6.2, 6.8)	98	7.1 (6.3, 7.9)	0.201	75	7.8 (6.8, 8.9)	0.027
	28-32 weeks	730	2.7 (2.5, 2.9)	92	2.9 (2.5, 3.5)	0.643	70	3.8 (3.1, 4.6)	0.005
	≥34 weeks	659	6.9 (6.3, 7.4)	82	8.9 (6.9, 11.2)	0.100	57	9.8 (7.4, 13.2)	0.044

BP, blood pressure; CI, confidence interval.

*Means (95%CI) were adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes; presented as geometric means.

†statistically significant difference between normal BP and elevated BP groups.

‡statistically significant difference between normal BP and hypertension group.

Higher levels of DBP and MAP in early pregnancy were significantly associated with higher log-transformed sFlt-1 values throughout pregnancy. Meanwhile, higher SBP levels were significantly associated with lower log-transformed PlGF levels both at 18-22 weeks (β= -0.02 per 10 mmHg SBP, *P*=0.011) and at 28-32 weeks (β= -0.02 per 10 mmHg SBP, *P*=0.031). Thus, rises in maternal SBP, DBP and MAP in the first trimester were significantly associated with higher sFlt-1/PlGF ratios during pregnancy (supplementary table 3).

Table 4 presents the significant association between blood pressure in early pregnancy and risks of preeclampsia (OR 2.5, 95% CI 1.5-4.0 per 10 mmHg SBP; OR 4.3, 95% CI 2.3-7.9 per 10 mmHg DBP; OR 4.1, 95% CI 2.2-7.7 per 10 mmHg MAP, respectively) after adjustment of potential confounders. Preterm preeclampsia was more closely associated with higher DBP than SBP (OR 6.0, 95% CI 2.3-7.9 per 10 mmHg DBP *vs.* OR 1.9, 95% CI 0.9-3.8 per 10 mmHg SBP).

Table 4. Logistic regression analysis for maternal blood pressure at 11-14weeks and adverse pregnancy outcomes.

Variable	Preeclampsia		Preterm preeclampsia		Term preeclampsia	
	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)
SBP (10mmHg)	3.0 (1.9, 4.6) †	2.5 (1.5, 4.0) †	1.9 (1.1, 3.5)	1.9 (0.9, 3.8)	4.1 (2.2, 7.8) †	3.2 (1.6, 6.4) †
DBP (10mmHg)	5.2 (2.9, 9.3) †	4.3 (2.3, 7.9) †	5.8 (2.4, 14.4) †	6.0 (2.3, 16.2) †	4.4 (2.1, 9.1) †	3.1 (1.4, 6.8) †
MAP (10mmHg)	5.1 (2.8, 9.0) †	4.1 (2.2, 7.7) †	5.1 (2.1, 11.9) †	5.6 (2.0, 15.5) †	4.7 (2.2, 9.7) †	3.3 (1.5, 7.4) †

OR, odds ratio; CI, confidence interval; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure

*Adjusted for maternal age, race, education, maternal body mass index at 11-14 weeks gestation and diabetes mellitus.

†$P < 0.01$.

Discussion

Our study confirmed that higher early pregnancy blood pressure levels were prospectively associated with increased risks of preeclampsia, including preterm and term preeclampsia. Furthermore, women with elevated blood pressure in early pregnancy already had a higher sFlt-1 level and sFlt-1/PlGF ratio in early gestation and throughout pregnancy. In contrast, PlGF levels in these women remained normal throughout gestation.

It is well established that women with chronic hypertension have several times the risk of preeclampsia than normotensive women.[14] However, the pathogensis is poorly understood and what role these angiogenic and anti-angiogenic factors play remains unclear. Although syncytiotrophoblast is a major source of sFlt-1 production, peripheral blood monocytes produce a small amount of sFlt-1 further stimulated by inflammation.[15,16] As chronic hypertension is often related to a chronic inflammatory status,[17] the slightly increased sFlt-1 level in hypertensive women in early pregnancy, as observed in our study, may reflect the chronic inflammatory status in early pregnancy. Our results showed that hypertensive women in early pregnancy might have an imbalanced angiogenic factors levels and such imbalanced angiogenic environment tended to continue during pregnancy, which might be associated with the increased risks of preeclampsia.

On the other hand, maternal circulating PlGF is highly expressed by the placenta

during pregnancy. It has both vasculogenic and angiogenic functions [18] and its level is likely to reflect the placental health conditions. For example, low PlGF concentrations preceding clinical onset of preeclampsia often occur in early-onset rather than late-onset preeclampsia.[19] Women who develop preeclampsia with fetal growth restriction (FGR) have further decreased PlGF levels compared with women who develop preeclampsia without FGR.[20-22] Early-onset preeclampsia and placenta-derived FGR are associated with placenta pathology such as incomplete remodeling of spiral arteries, acute atherosis and thrombosis in spiral arteries and syncytiotrophoblast necrosis.[23,24] In our study, women with elevated blood pressure in early pregnancy, the PlGF level remained by and large normal throughout pregnancy and newborn's birth weight was not significantly different among the three groups. Thus, our findings seem to suggest that the placental implantation and development might not be impaired in these women.

As poor placentation is not a unique cause of developing preeclampsia, enhanced placental oxidative and endoplasmic reticulum stress and increased maternal systemic inflammatory responses are thought to play crucial roles in preeclampsia as well.[25,26] Thus, preexisting endothelial dysfunction in hypertensive women could be exacerbated as a result of physiological burden of pregnancy even without an abnormal placentation.[27] Taking all things considered, we propose that the imbalanced angiogenic factors environment and, perhaps more importantly, preexisting endothelial susceptibility and dysfunction, may play a critical role in the development

of preeclampsia in women with elevated blood pressure in early pregnancy.

To our best knowledge, this was the first prospective cohort study that illustrated the dynamic changes of angiogenic and anti-angiogenic factors throughout pregnancy in women with different blood pressure status in early pregnancy. The NORA cohort was a well-performed prospective study with comprehensive information including clinical, biophysical and biochemical markers. The follow-up rate was 99.1% (926/934) at the end of pregnancy. Measurements of blood pressure and serum angiogenic factors were performed according to standardized protocols. In our study, three women reported using antihypertensive medications during pregnancy; thus, the results should not be affected by the medication issue. On the other hand, as most of our participants were low-risk pregnant women, our results may not be applicable to high-risk women. As it was an observational study, the potential residual confounding and selection bias might have some impacts on our results.

Conclusion

Women with elevated blood pressure in early pregnancy already had a higher sFlt-1/PlGF ratio in early gestation and throughout pregnancy, and an increased risk of preeclampsia. In contrast, PlGF levels in these women remained normal throughout gestation. Our findings suggest that the imbalanced angiogenic factors levels throughout gestation might play a crucial role in developing preeclampsia in women with preexisting elevated blood pressure. Our study also supports that preconception

or early pregnancy high blood pressure, defined as SBP≥130 mmHg or DBP≥80 mmHg according to 2017 ACC/AHA guideline, should cause clinical awareness both during pregnancy and in their later life.

Acknowledgments

We thank all the NORA cohort (Neonatal and Obstetrics Risks Assessment) participants for their commitment to the study, the NORA cohort investigators and staff.

Author Contributions

JZ: performed the statistical analysis, searched literature and drafted the manuscript. JZ (corresponding author): had the original idea, provided guidance for the statistical analysis and revised the manuscript. MJN, BC, GSY and KHT: participated in the data collection, reviewed and revised the manuscript.

Funding: The authors received no finical support for the research, authorship, and/or publication of this article.

Competing interests: None declared.

Patient consent for publication: Obtained.

Ethics approval: This study was approved by the SingHealth Centralised Institutional Review Board Ethics Committee, Singapore (CIRB Ref No. 2010/214/D).

Provenance and peer review: Not commissioned; externally peer reviewed.

Data availability statement: All data relevant to the study are included in the article or uploaded as supplementary information.

References

1. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A* 1993; 90:10705-10709.
2. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, *et al*. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; 350(7): 672-83.
3. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, *et al.* Predictive Value of the sFlt-1: PlGF Ratio in Women with Suspected Preeclampsia. N E*ngl J Med* 2016 Jan 7;374(1):13-22.
4. NICE. PlGF-based Testing to Help Diagnose Suspected Pre-eclampsia (Triage PlGF Test, Elecsys Immunoassay sFlt-1/PlGF Ratio, DELFIA Xpress PlGF 1-2-3 Test, and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio). Diagnostics Guidance 23. London: NICE, 2016.
5. 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. *J Clin Invest* 2003;111(5):649-58.
6. Sela S, Itin A, Natanson-Yaron S, Greenfield C, Goldman-Wohl D, Yagel S, *et al.* A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-type-specific splicing and implications to vascular endothelial growth factor homeostasis and preeclampsia. *Circ Res* 2008;102(12):1566-74.
7. Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem* 1997;272(38):23659-67.
8. Warrington JP, George EM, Palei AC, Spradley FT, Granger JP. Recent advances in the understanding of the pathophysiology of preeclampsia. *Hypertension* 2013;62(4):666-73.
9. Yang J, Pearl M, DeLorenze GN, Romero R, Dong Z, Jelliffe-Pawlowski L, *et al.*

Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. *Am J Obstet Gynecol.* 2016; 215(3): 359.e1-9.

10. Ng QJ, Zhang J, Dai F, Ng MJ, Razali NS, Win NM, *et al.* Neonatal and Obstetric Risk Assessment (NORA) Pregnancy Cohort Study in Singapore. *Int J Gynaecol Obstet* 2018; 4(1): 31-37.
11. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, *et al.* Recommendations for blood pressure measurement in humans and experimental animals. *Circulation* 2005; 111:697-716.
12. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; 20(1): IX-XIV.
13. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, *et al.* 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018;71(6):1269-1324.
14. Bartsch E, Medcalf KE, Park AL, Ray JG; High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. *BMJ* 2016;353: i1753.
15. Rajakumar A, Michael HM, Rajakumar PA, Shibata E, Hubel CA, Karumanchi SA, *et al.* Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. *Placenta* 2005;26(7):563-73.
16. Freeman DJ, McManus F, Brown EA, Cherry L, Norrie J, Ramsay JE, *et al.* Short- and long-term changes in plasma inflammatory markers associated with

preeclampsia. *Hypertension* 2004;44(5):708-14.

17. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *Biomed Res Int* 2014; 2014: 406960.
18. De Falco S. The discovery of placenta growth factor and its biological activity. *Exp Mol Med* 2012;44(1):1-9.
19. McElrath TF, Lim KH, Pare E, Rich-Edwards J, Pucci D, Troisi R, *et al.* Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. *Am J Obstet Gynecol* 2012;207(5): 407.e1-7.
20. Powers RW, Roberts JM, Plymire DA, Pucci D, Datwyler SA, Laird DM, *et al.* Low placental growth factor across pregnancy identifies a subset of women with preterm preeclampsia: type 1 versus type 2 preeclampsia? *Hypertension* 2012;60(1):239-46.
21. 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. *J Matern Fetal Neonatal Med* 2008;21(1):9-23.
22. Chaiworapongsa T, Romero R, Whitten AE, Korzeniewski SJ, Chaemsaithong P, Hernandez-Andrade E, *et al.* The use of angiogenic biomarkers in maternal blood to identify which SGA fetuses will require a preterm delivery and mothers who will develop pre-eclampsia. *J Matern Fetal Neonatal Med* 2016;29(8):1214-28.
23. Nelson DB, Ziadie MS, McIntire DD, Rogers BB, Leveno KJ. Placental pathology suggesting that preeclampsia is more than one disease. *Am J Obstet Gynecol* 2014;210(1): 66.e1-7.
24. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. *Am J Obstet Gyneco.* 2018;218(2S): S745-S761.
25. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet*

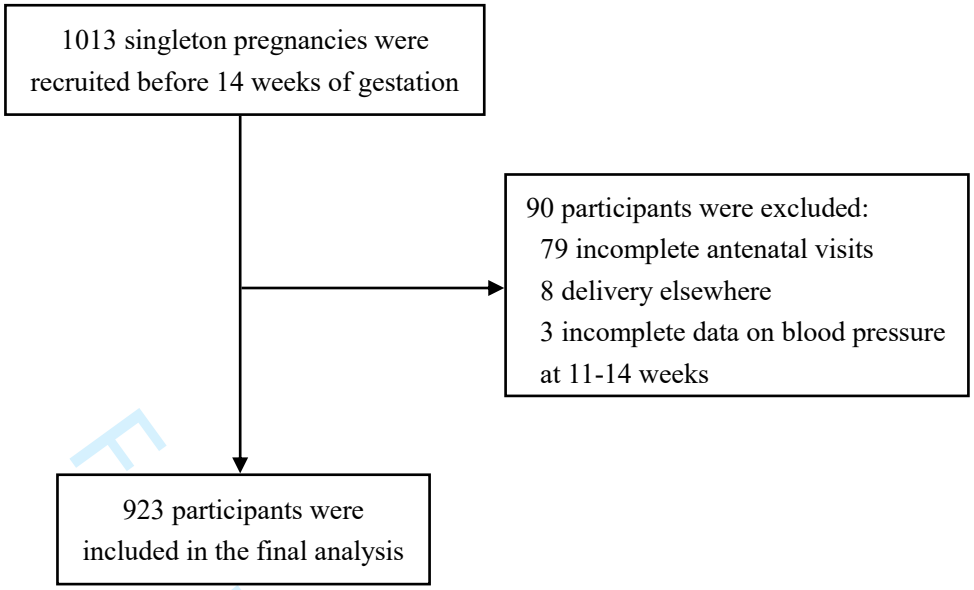
2010;376(9741):631-44.

26. Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? *Placenta* 2014;35 Suppl: S20-5.

27. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am J Obstet Gynecol* 1996;175(5):1365-70.

Figure legends

Figure 1. Flowchart of participants.



Supplementary table 1. Logarithm-transformed maternal serum angiogenic factors levels at 4 time points during pregnancy by maternal blood pressure at 11-14weeks in the NORA cohort.

Angiogenic factors	Time points	Normal BP		Elevated BP				Hypertension (stage 1 and stage 2)			
		N	mean (95%CI) *	N	mean (95%CI) *	Mean difference (95%CI) *†	*P* value †	N	mean (95%CI) *	Mean difference (95%CI) *‡	*P* value ‡
sFlt-1 (pg/ml)	11-14 weeks	746	3.20 (3.19, 3.21)	98	3.24 (3.20, 3.27)	0.04 (-0.01, 0.08)	0.096	75	3.24 (3.20, 3.29)	0.05 (-0.01, 0.10)	0.079
	18-22 weeks	745	3.23 (3.22, 3.25)	98	3.24 (3.20, 3.29)	0.01 (-0.04, 0.06)	0.824	75	3.28 (3.23, 3.33)	0.05 (-0.01, 0.11)	0.139
	28-32 weeks	730	3.22 (3.20, 3.23)	92	3.23 (3.19, 3.27)	0.01 (-0.04, 0.06)	0.868	70	3.31 (3.26, 3.36)	0.09 (0.03, 0.15)	0.001
	≥34 weeks	659	3.41 (3.40, 3.43)	82	3.45 (3.41, 3.50)	0.04 (-0.02, 0.10)	0.201	57	3.52 (3.47, 3.58)	0.11 (0.04, 0.17)	0.000
PlGF (pg/ml)	11-14 weeks	746	1.57 (1.56, 1.58)	98	1.54 (1.50, 1.58)	-0.03 (-0.08, 0.01)	0.243	75	1.55 (1.50, 1.59)	-0.02 (-0.07, 0.03)	0.593
	18-22 weeks	745	2.43 (2.41, 2.44)	98	2.39 (2.35, 2.43)	-0.03 (-0.08, 0.02)	0.261	75	2.39 (2.34, 2.44)	-0.03 (-0.09, 0.03)	0.364
	28-32 weeks	730	2.79 (2.77, 2.81)	92	2.76 (2.70, 2.83)	-0.02 (-0.10, 0.05)	0.718	70	2.73 (2.66, 2.80)	-0.06 (-0.14, 0.03)	0.270
	≥34 weeks	659	2.58 (2.55, 2.60)	82	2.51 (2.43, 2.58)	-0.07 (-0.17, 0.02)	0.178	57	2.53 (2.44, 2.63)	-0.05 (-0.16, 0.07)	0.585
sFlt-1/ PlGF ratio	11-14 weeks	746	1.63 (1.61, 1.65)	98	1.70 (1.65, 1.74)	0.07 (0.01, 0.13)	0.013	75	1.70 (1.64, 1.75)	0.07 (0.01, 0.13)	0.042
	18-22 weeks	745	0.81 (0.79, 0.83)	98	0.85 (0.80, 0.90)	0.05 (-0.02, 0.11)	0.201	75	0.89 (0.83, 0.95)	0.08 (0.01, 0.15)	0.027

28-32 weeks	730	0.43 (0.40, 0.46)	92	0.46 (0.39, 0.54)	0.03 (-0.06, 0.13)	0.643	70	0.58 (0.49, 0.66)	0.15 (0.04, 0.25)	0.005
≥34 weeks	659	0.84 (0.80, 0.87)	82	0.95 (0.84, 1.05)	0.11 (-0.02, 0.24)	0.100	57	0.99 (0.87, 1.12)	0.16 (0.01, 0.31)	0.044

BP, blood pressure; CI, confidence interval.

*Means (95%CI) were adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes;

†statistically significant difference between normal BP and elevated BP groups.

‡statistically significant difference between normal BP and hypertension group.

Supplementary table 2. Maternal serum angiogenic factors levels during pregnancy by preeclampsia in women with different BP status in early pregnancy.

Angiogenic factors	Time points	Normal BP			Elevated BP			Hypertension (stage 1 and stage 2)		
		PE (n=6)	Non-PE (n=744)	*P* value	PE (n=3)	Non-PE (n=95)	*P* value	PE (n=12)	Non-PE (n=63)	*P* value
		mean (95%CI) *	mean (95%CI) *		mean (95%CI) *	mean (95%CI) *		mean (95%CI) *	mean (95%CI) *	
sFlt-1 (pg/ml)	11-14 weeks	1514 (1096, 2089)	1622 (1549, 1660)	0.718	1778 (1096, 2884)	1622 (1514, 1778)	0.756	1349 (1122, 1622)	1660 (1549, 1820)	0.048
	18-22 weeks	1698 (1175, 2512)	1738 (1660, 1778)	0.925	1479 (813, 2692)	1660 (1514, 1862)	0.692	1738 (1288, 2291)	1738 (1514, 1995)	0.960
	28-32 weeks	3090 (2188, 4365)	1698 (1622, 1738)	<0.001	1995 (1148, 3467)	1549 (1413, 1698)	0.388	3020 (2188, 4074)	1622 (1445, 1862)	<0.001
	⩾34 weeks	6026 (3548, 10233)	2630 (2512, 2692)	0.002	3715 (2042, 6607)	2692 (2399, 2951)	0.295	6026 (3802, 9550)	2951 (2570, 3388)	0.005
PlGF (pg/ml)	11-14 weeks	29.5 (21.4, 41.7)	37.2 (36.3, 38.0)	0.199	32.4 (20.0, 53.7)	36.3 (33.1, 38.9)	0.716	24.6 (19.1, 30.9)	35.5 (32.4, 39.8)	0.005
	18-22 weeks	208.9 (144.5, 309.0)	269.2 (263.0, 281.8)	0.191	182.0 (114.8, 288.4)	239.9 (218.8, 257.0)	0.257	151.4 (120.2, 190.6)	234.4 (213.8, 263.0)	0.001
	28-32 weeks	269.2 (158.5, 457.1)	631.0 (602.6, 660.7)	0.002	323.6 (166.0, 645.7)	562.3 (501.2, 645.7)	0.118	182.0 (120.2, 269.2)	588.8 (489.8, 691.8)	<0.001
	⩾34 weeks	169.8 (67.6, 426.6)	380.2 (363.1, 407.4)	0.083	208.9 (85.1, 512.9)	316.2 (269.2, 371.5)	0.372	147.9 (66.1, 323.6)	323.6 (257.0, 416.9)	0.059
sFlt-1/ PlGF ratio	11-14 weeks	50.1 (33.1, 77.6)	42.7 (41.7, 44.7)	0.454	53.7 (28.8, 100.0)	45.7 (40.7, 50.1)	0.591	56.2 (42.7, 74.1)	46.8 (41.7, 52.5)	0.235

18-22 weeks	8.1	(5.0, 12.9)	6.5 (6.2, 6.6)	0.323	8.1	(4.2, 15.9)	7.1 (6.3, 7.9)	0.674	11.2	(7.9, 16.2)	7.4 (6.3, 8.5)	0.029
28-32 weeks	11.5	(6.0, 21.9)	2.7 (2.5, 2.8)	<0.001	6.0	(2.4, 15.1)	2.8 (2.4, 3.2)	0.093	16.6	(9.8, 28.2)	2.8 (2.2, 3.6)	<0.001
⩾34 weeks	35.5	(10.5, 120.2)	6.8 (6.3, 7.4)	0.008	17.8	(4.9, 64.6)	8.5 (6.8, 10.7)	0.269	40.7	(14.5, 114.8)	9.1 (6.6, 12.6)	0.008

BP, blood pressure; CI, confidence interval; PE, preeclampsia.

*Means (95%CI) were adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test from models with logarithm-transformed serum angiogenic factors levels as outcomes; presented as geometric means.

Supplementary table 3. Associations of maternal blood pressure at 11-14weeks with serum angiogenic factors levels during pregnancy.

Variable	Time points	Log sFlt-1		Log PlGF		Log sFlt-1/ PlGF ratio	
		β (95% CI) *	*P* value	β (95% CI) *	*P* value	β (95% CI) *	*P* value
Systolic blood pressure	11-14 weeks	0.01 (0.004, 0.03)	0.009	-0.01 (-0.02, 0.0004)	0.057	0.03 (0.01, 0.04)	< 0.001
(10mmHg)	18-22 weeks	0.01 (-0.01, 0.02)	0.227	-0.02 (-0.03, -0.004)	0.011	0.02 (0.01, 0.04)	0.003
	28-32 weeks	0.01 (-0.001, 0.02)	0.068	-0.02 (-0.04, -0.002)	0.031	0.03 (0.01, 0.06)	0.007
	⩾34 weeks	0.02 (0.01, 0.03)	0.009	-0.02 (-0.05, 0.001)	0.059	0.04 (0.01, 0.07)	0.011
Diastolic blood pressure	11-14 weeks	0.02 (0.01, 0.04)	0.002	-0.01 (-0.03, 0.001)	0.069	0.04 (0.02, 0.05)	< 0.001
(10mmHg)	18-22 weeks	0.02 (0.004, 0.04)	0.014	-0.01 (-0.02, 0.01)	0.388	0.03 (0.01, 0.05)	0.008
	28-32 weeks	0.04 (0.02, 0.05)	< 0.001	-0.02 (-0.04, 0.004)	0.104	0.05 (0.03, 0.08)	< 0.001
	⩾34 weeks	0.04 (0.02, 0.06)	< 0.001	-0.02 (-0.05, 0.01)	0.134	0.06 (0.02, 0.10)	0.003
Mean arterial pressure	11-14 weeks	0.02 (0.01, 0.04)	0.002	-0.02 (-0.03, -0.001)	0.043	0.04 (0.02, 0.06)	< 0.001
(10mmHg)	18-22 weeks	0.02 (0.001, 0.03)	0.044	-0.01 (-0.02, 0.01)	0.506	0.02 (0.002, 0.04)	0.030
	28-32 weeks	0.03 (0.01, 0.04)	0.001	-0.02 (-0.05, 0.001)	0.058	0.05 (0.02, 0.08)	0.001
	⩾34 weeks	0.04 (0.02, 0.05)	< 0.001	-0.03 (-0.06, -0.003)	0.029	0.07 (0.03, 0.11)	< 0.001

Abbreviations: CI, confidence interval.

*Adjusted for maternal race, smoking during pregnancy, maternal body mass index at blood test and gestational weeks at blood test.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 2, line 9
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5, line 14-22
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 6, line 1-4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 6, line 8-9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 6, line 8-22
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 7, line 2-14
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 7, line 16 ~ Page 8, line 12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 8, line 14 ~ Page 9, line 10
Bias	9	Describe any efforts to address potential sources of bias	Page 7, line 4-7
Study size	10	Explain how the study size was arrived at	Page 7, line 7-14
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8, line 6-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 9, line 12~ Page 10, line 10
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 10, line 17-20
		(b) Give reasons for non-participation at each stage	Page 7,

			line 8-9
		(c) Consider use of a flow diagram	Page 7, figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 11, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Page 15, Table 3
		(c) Summarise follow-up time (eg, average and total amount)	Page 15, Table 3
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 15, Table 3
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 15, Table 3, Page 17, Table 4
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 18, line 2-7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 20, line 9-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 20, line 17-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 20, line 11-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 22, Line 12-13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.