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FIRST TRIMESTER SERUM ANGIOGENIC AND ANTI-ANGIOGENIC FACTORS IN WOMEN WITH CHRONIC HYPERTENSION FOR THE PREDICTION OF PREECLAMPSIA

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CONDENSATION

Women with chronic hypertension and superimposed preeclampsia have lower first trimester serum PLGF and sFLT-1 when compared to those without superimposed preeclampsia and normotensive controls.

Short version of article title: Chronic hypertension: first trimester serum PLGF and sFLT-1 in the prediction for preeclampsia.

AJOG AT A GLANCE:

A. Why was the study conducted?

To investigate whether in women with chronic hypertension first-trimester serum PLGF and sFLT-1 are different between those with superimposed preeclampsia and those without and to compare these values to those in normotensive controls.

B. What are the key findings?

Women with chronic hypertension and superimposed preeclampsia have lower first trimester serum PLGF and sFLT-1 when compared to those without superimposed preeclampsia and normotensive controls. These differences are modest and the ROC curve for prediction of preeclampsia is poor.

C. What does the study add to what is known?

In the general obstetric population, first trimester serum PLGF is a significant predictor of preeclampsia but evidence for sFLT-1 is conflicting. This study adds that in women with chronic hypertension neither parameter is useful for the prediction of superimposed preeclampsia.

ABSTRACT

Background: An imbalance between angiogenic and antiangiogenic factors is thought to be a central pathogenetic mechanism in preeclampsia. In pregnancies that subsequently develop preeclampsia the maternal serum concentration of the angiogenic placental growth factor (PLGF) is decreased from as early as the first trimester of pregnancy and the concentration of the antiangiogenic soluble fms-like tyrosine kinase-1 (sFLT-1) is increased in the last few weeks before the clinical presentation of the disease. Chronic hypertension, which complicates 1-2% of pregnancies, is the highest risk factor for development of preeclampsia among all other factors in maternal demographic characteristics and medical history. Two previous studies in women with chronic hypertension reported that first-trimester serum PLGF and sFLT-1 were not significantly different between those that developed superimposed preeclampsia and those that did not, whereas a third study reported that concentrations of PLGF were decreased.

Objective: To investigate whether in women with chronic hypertension serum concentrations of PLGF and sFLT-1 and sFLT-1/PLGF ratio at 11^{+0} to 13^{+6} weeks' gestation are different between those that developed superimposed preeclampsia and those that did not and to compare these values to those in normotensive controls.

Study design: The study population comprised of 650 women with chronic hypertension, including 202 that developed superimposed preeclampsia and 448 that did not develop preeclampsia, and 142 normotensive controls. Maternal serum concentration of PLGF and sFLT-1 were measured by an automated biochemical

analyzer and converted into multiples of the expected median (MoM) using multivariate regression analysis in the control group. Comparisons of PLGF, sFLT-1 and sFLT-1/PLGF ratio in MoM values between the two groups of chronic hypertension and the controls were made by the ANOVA or the Kruskal-Wallis test.

Results: In the group of chronic hypertension that developed preeclampsia, compared to those that did not develop preeclampsia, there were significantly lower median concentrations of serum PLGF MoM (0.904, interquartile range (IQR) 0.771-1.052 vs. 0.948, IQR 0.814-1.093; p=0.014) and sFLT-1 MoM (0.895, IQR 0.760-1.033 vs 0.938, IQR 0.807-1.095; p=0.013) and they were both lower than in the normotensive controls (1.009, IQR 0.901-1.111 and 0.991, IQR 0.861-1.159, respectively; P<0.01 for both). There were no significant differences between the three groups in sFLT-1 / PLGF ratio. In women with chronic hypertension serum PLGF and sFLT-1 provided poor prediction of superimposed preeclampsia (area under the curve 0.567, 95% confidence interval (CI) 0.537-0.615 and 0.546, 95% CI 0.507-0.585, respectively).

Conclusions: Women with chronic hypertension, and particularly those who subsequently developed preeclampsia, have reduced first trimester concentrations of both PLGF and sFLT-1.

INTRODUCTION

An imbalance between angiogenic and antiangiogenic factors is thought to be a central pathogenetic mechanism in preeclampsia.[1-3] Extensive studies have established that the maternal serum concentration of the angiogenic placental growth factor (PLGF) is decreased from as early as the first trimester of pregnancies later complicated by preeclampsia; therefore, PLGF has since been incorporated into algorithms aimed at the prediction of preeclampsia at different stages in pregnancy.[4-12] The antiangiogenic soluble fms-like tyrosine kinase-1 (sFLT-1) is increased in the last few weeks before the clinical presentation of preeclampsia.[13-16] However, the evidence concerning first trimester serum sFLT-1 in pregnancies that subsequently develop preeclampsia is contradictory, with some studies reporting increased [17,18] or decreased[19,20] concentrations and others no significant difference from normotensive pregnancies.[14,21-23]

Chronic hypertension, which complicates 1-2% of pregnancies, is the highest risk factor for development of preeclampsia among all other maternal demographic characteristics and medical history.[24-26] In large prospective studies of women recruited from the first trimester, superimposed preeclampsia complicated 23% of pregnancies with chronic hypertension and, after adjustment for confounding factors, the risk of both preterm and term preeclampsia was 5-6 times higher in women with chronic hypertension than in those without chronic hypertension.[24-26] In women without chronic hypertension that develop preeclampsia, particularly preterm preeclampsia, there is evidence of impaired placentation with consequent reduced placental perfusion, oxidative stress and release of trophoblast-derived factors into the maternal circulation. This triggers generalized endothelial dysfunction and an

exaggerated inflammatory response that underlines many of the signs and symptoms of the disease.[1,27,28]

In women with chronic hypertension, there is endothelial dysfunction even before pregnancy and it was proposed that, in such cases, preeclampsia can develop in the absence of impaired placentation; the pre-existing endothelial dysfunction is exacerbated by the physiological burden of pregnancy, as normal pregnancies carry a low-grade systemic inflammatory response.[29-31] One previous study reported that, in women with chronic hypertension and superimposed preeclampsia, serum PLGF at 11-13 weeks' gestation was reduced but the degree of deviation from normal was less than in women without chronic hypertension who developed preeclampsia.[32] Two other studies reported that serum PLGF and sFLT-1 at 12-15[33] or at 11-27[34] weeks' gestation in women with chronic hypertension were not significantly different between those that developed superimposed preeclampsia and those that did not.[33,34] These results are contrary to studies of women with chronic hypertension and superimposed preeclampsia in the third trimester, which have findings more consistent to the general obstetric population with a raised sFLT-1.[35,36]

The objective of this study was to investigate whether in women with chronic hypertension serum concentrations of PLGF and sFLT-1 and sFLT-1/PLGF ratio at 11^{+0} to 13^{+6} weeks' gestation are different between those that developed superimposed preeclampsia and those that did not and to compare these values to those in normotensive controls.

MATERIALS & METHODS

Study population

This was a case control study involving analysis of serum PLGF and sFLT-1 in stored samples obtained at 11-13 weeks' gestation from 650 singleton pregnancies complicated by chronic hypertension and 142 normotensive controls attending for pregnancy care at King's College Hospital, London, UK, between January 2011 and September 2018. The visit at 11-13 weeks is offered routinely to all women attending our hospital for pregnancy care and includes first, recording of maternal demographic characteristics and medical history, second measurement of maternal weight and height, third, measurement of blood pressure using an automated device validated for use in pregnancy and preeclampsia,[37] fourth, ultrasound scan to determine gestational age from the crown-rump length, examine the fetal anatomy and measure fetal nuchal translucency, and fifth, measurement of serum free β-hCG and PAPP-A as part of screening for trisomies. Women agreeing to participate in research provide a venous blood sample and serum is stored at -80°C. The women provided written informed consent to participate in the study, which was approved by the National Health Service Research Ethics Committee.

Assay analysis

Maternal serum concentration of PLGF and sFLT-1 in pg/mL were measured using thawed samples by an automated biochemical analyzer (BRAHMS KRYPTOR compact PLUS, Thermo Fisher Scientific, Hennigsdorf, Germany). In this analyzer

the inter-assay coefficients of variation for the low and high concentrations were 22% and 5% for PLGF, and 5% and 5% for sFLT-1, respectively; assays cover a measurement range from 3.6 to 7,000 pg/mL for PLGF and from 22 to 90,000 pg/mL for sFLT-1.

Inclusion and exclusion criteria

The inclusion criteria for this study were singleton pregnancies resulting in the live birth or stillbirth of non-malformed babies at $\geq$24 weeks' gestation. We excluded pregnancies with fetal aneuploidies or major defects diagnosed antenatally or in the neonatal period and pregnancies ending in miscarriage at <24 weeks' gestation. For every five cases with chronic hypertension we selected approximately one control from uncomplicated pregnancies that resulted in the live birth of phenotypically normal neonates and were matched to the cases for storage time of maternal serum and racial origin, because the incidence of chronic hypertension is three times higher in Black than White women.[25] As there was no existing literature to guide a power analysis, we performed an interim power calculation that demonstrated that 131 controls and 624 women with chronic hypertension would provide a type I error (alpha) of 0.01 and a type II error (beta) of 0.05.

Diagnosis and management of chronic hypertension

Women are classified as chronic hypertension if they have pre-pregnancy hypertension or have BP $\geq$140/90 mmHg on two consecutive clinical visits prior to 20 weeks' gestation. At their booking visit, all women underwent screening for pre-

existing renal and liver disease. Women with pre-existing renal or liver disease or those with proteinuria and / or creatinine[38,39] or transaminases[40] above the 95th centile for gestation were excluded from the study in order to avoid bias in the diagnosis of preeclampsia. In women with chronic hypertension our policy is to maintain the BP at 130-140/80-90 mmHg throughout pregnancy with the use of antihypertensive medication; these medications are stopped or reduced if the BP falls <130/80 mmHg on two consecutive visits.

Definitions of adverse pregnancy outcomes

Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) as the presence of hypertension along with at least one of the following: renal involvement (proteinuria ≥300 mg/24h and/or serum creatinine ≥90 µmol/L or 1 mg/dL), liver impairment (serum transaminases >70 IU/L), neurological complications (e.g. eclampsia), thrombocytopenia (platelet count <150,000/µL), uteroplacental insufficiency with fetal growth restriction.[41] In addition, preeclampsia was subdivided according to gestational age at diagnosis into preterm preeclampsia with onset at <37 weeks' gestation and term preeclampsia with onset at ≥37 weeks. Severe hypertension was defined by the presence of systolic BP ≥160 mmHg and / or diastolic BP ≥110 mmHg. Small for gestational age (SGA) was defined as birth weight <10th percentile without adjustment for maternal characteristics.[42]

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Statistical analysis

Numerical data were expressed as mean (standard deviation) or median and interquartile range (IQR) for normally and non-normally distributed data, respectively.

Normality of continuous variables was assessed by the Kolmogorov-Smirnov test. The distribution of sFLT-1, PLGF and sFLT-1/PLGF ratio were logarithmically transformed to approximate Gaussian distribution. Log sFLT-1, log PLGF and log sFLT-1/PLGF were converted into multiples of the expected median (MoM) using multivariate regression analysis in the control group to determine which of the maternal characteristics were significant predictors of log sFLT-1, log PLGF and log sFLT-1/PLGF ratio. Between group comparisons were made by the ANOVA or the Kruskal-Wallis test, with Bonferroni correction for post-hoc analysis, for normally and non-normally distributed data, respectively. The Chi-square test was used to compare categorical variables. The performance of MoM log sFLT-1 and MoM log PLGF for the prediction of preeclampsia was assessed by univariate logistic regression and the Receiver Operating Characteristic Curve (ROC curve).

Statistical analysis was performed using SPSS (Version 24; SPSS Inc, Chicago,IL).

RESULTS

Population characteristics

The inclusion criteria for women with chronic hypertension were met by 650 pregnancies and these included 448 that did not develop superimposed

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preeclampsia and 202 that developed preeclampsia. The control group consisted of 142 normotensive women. Maternal and pregnancy characteristics of the three groups are compared in Table 1.

In the two chronic hypertension groups, compared to controls, maternal age and systolic and diastolic blood pressure at 11-13 weeks' gestation were higher (Table 1). In the group of chronic hypertension that developed preeclampsia, compared to those that did not develop preeclampsia, the incidence of previous preeclampsia, family history of preeclampsia and use of antihypertensive medications at 11-13 weeks' gestation was higher.

In the two chronic hypertension groups, compared to controls, median gestational age at delivery and median birthweight percentile were lower and incidence of SGA neonates was higher (Table 1). In the group of women with chronic hypertension that developed preeclampsia, compared to those that did not develop preeclampsia, there was a higher incidence of treatment with antihypertensive drugs at the time of delivery, development of severe hypertension and delivery of SGA neonates and lower median gestational age at delivery and median birthweight percentile.

Serum PLGF and sFLT-1 and sFLT-1 / PLGF ratio

Creation of multiples of the median using data from controls

In the multiple regression model for log sFLT-1, significant independent contributions were provided by age, weight, Black racial origin, parity and history of previous preeclampsia (p= <0.001, R^2= 0.251): Log sFLT-1 expected = 3.142305 + 0.007276

x age (in years) − 0.003325 x weight (in kg) + 0.102288 x Black racial origin − 0.172146 x multiparity + 0.158197 x previous preeclampsia.

In the multiple regression model for log PLGF, significant independent contributions were provided by age, weight and Black racial origin (p= <0.001, R^2= 0.207): Log PLGF expected = 1.524666 + 0.008217 x age (in years) − 0.002721 x weight (in kg) + 0.170813 x Black racial origin.

In the multiple regression model for log sFLT-1/PLGF ratio, significant independent contributions were provided by parity alone (p= 0.001, R^2= 0.082): Log sFLT-1/PLGF ratio expected = 1.486950 - 0.151459 if parous.

Comparison of multiples of the median between groups

In the two chronic hypertension groups, compared to controls, both mean PLGF and mean sFLT-1 were lower (Table 2, Figure 1). In the group of women with chronic hypertension that developed preeclampsia, compared to those that did not develop preeclampsia, both mean PLGF and mean sFLT-1 were lower (Table 1, Figure 1). There were no significant differences between the three groups in sFLT-1/PLGF ratio. Subgroup analysis of women with chronic hypertension showed no significant difference between preterm and term preeclampsia in PLGF MoM (0.86 and 0.91 MoM, respectively, p=0.47), sFLT-1 MoM (0.88 and 0.90 MoM, respectively, p=1.00), or sFLT-1/PLGF ratio (1.02 and 0.99, respectively, p=1.00).

Performance of screening

Univariate logistic regression analysis demonstrated that both MoM log sFLT-1 and MoM log PLGF were predictors of the development of preeclampsia, albeit with a low Nagelkerke R-square. More specifically, the logistic regression model for sFLT-1 was

logit preeclampsia = -1.18-1.39* MOM log sFLT1-1 (p<0.0001, Nagelkerke R-square 0.02) and for PLGF logit preeclampsia = -1.19-1.56* MOM log PLGF (p<0.0001, Nagelkerke R-square 0.03).

Similarly, the area under the curve for the ROC curves was small (area under the curve 0.567, 95% confidence interval (CI) 0.537-0.615 for PLGF and 0.546, 95% CI 0.507-0.585 for sFLT-1) (Figure 2).

DISCUSSION

Principal findings of the study

The findings of this study demonstrate that first, in women with chronic hypertension, compared to controls, serum concentrations of PLGF and sFLT-1 at 11-13 weeks' gestation are lower but the sFLT-1/PLGF ratio was not significantly different, second, within the group of women with chronic hypertension serum concentrations of PLGF and sFLT-1 were lower in those that developed superimposed preeclampsia compared to those that did not develop preeclampsia, and third, in women with chronic hypertension first trimester serum PLGF and sFLT-1 provide poor prediction of superimposed preeclampsia.

The study has also confirmed that a high proportion of women with chronic hypertension develop superimposed preeclampsia and in these pregnancies there is more severe hypertension and need for antihypertensive medications, earlier gestational age at delivery and higher incidence of SGA neonates.

Comparison with findings of previous studies

Previous first-trimester screening studies in general populations of pregnant women reported that in those that subsequently develop preeclampsia serum PLGF is reduced and algorithms incorporating PLGF have been used for first-trimester prediction of subsequent development of preeclampsia.[4,7-9] Two previous studies in women with chronic hypertension reported no significant differences in serum PLGF at 12-15[33] or at 11-27[34] weeks' gestation between those that subsequently

developed superimposed preeclampsia compared to those that did not develop preeclampsia; in these studies there were no normotensive controls.[33,34] Another study reported that in women with chronic hypertension those that develop superimposed preeclampsia have reduced first-trimester concentrations of serum PLGF but the decrease is less than in women without chronic hypertension that develop preeclampsia.[32] The results of our study demonstrate that in women with chronic hypertension, irrespective of whether they develop superimposed preeclampsia or not, first-trimester serum PLGF is lower than in normotensive controls and the difference is more marked in those that develop preeclampsia; however, within the group with chronic hypertension PLGF provides poor prediction of preeclampsia.

We also found that in women with chronic hypertension, compared to normotensive controls, first-trimester sFLT-1 is reduced and the reduction is greater in those that develop superimposed preeclampsia. In two previous studies in women with chronic hypertension there was no significant difference in serum sFLT-1 between those that developed superimposed preeclampsia and those that did not. [33,34] Studies in general populations reported that first-trimester sFLT-1 in women that subsequently develop preeclampsia may be decreased,[19,20] increased[17,18] or not significantly different from those in normotensive pregnancies.[14,21-23]

In non-pregnant populations extensive studies have investigated the association between angiogenic factors and cardiovascular disease,[43] and three of the studies were focused in women with chronic hypertension.[44-46] Two of these studies reported lower sFLT-1 concentrations in patients with chronic hypertension, when compared to normotensive controls, and inverse correlation between sFLT-1 concentrations and cardiovascular risk.[44,45] In contrast, the third study reported that

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in patients with chronic hypertension, when compared to normotensive controls, serum sFLT-1 concentrations were increased but there was a substantial reduction after commencement of anti-hypertensive medication.[46]

A possible explanation for discrepancies in reported results of sFLT-1 could be that populations with chronic hypertension are heterogeneous and many factors such as antihypertensive treatment and BP control could influence the concentrations of angiogenic markers.

Interpretation of results

In normal pregnancy, serum PLGF increases with gestational age to reach a peak at around 30 weeks and decreases thereafter until delivery; in contrast, the concentrations of serum sFLT-1 remain low and relatively constant until about 30 weeks, but increase exponentially thereafter until delivery.[14] In pregnancies that develop preeclampsia serum PLGF is decreased throughout pregnancy, whereas sFLT-1 increases within 3-5 weeks prior to the development of the clinical signs of the disease.[4-14]

In normal pregnancy, placentation occurs in an environment of relative hypoxia which up-regulates production of proangiogenic VEGF and down-regulates the early production of PLGF.[47-49] Production of the antiangiogenic sFLT-1 in the first trimester is a physiological response to counteract the overspill of VEGF into the maternal circulation.[50,51] With advancing gestational age and improved placental oxygenation, production of VEGF and consequently sFLT-1 remains low but production of PLGF increases. It is possible that during the third trimester of normal

pregnancy the increased demands of the growing fetus may result in relative placental hypoxia with consequent increase in sFLT-1 and decrease in PLGF.

In pregnancies that develop preeclampsia there is impaired placentation and it could be anticipated that the degree of early placental hypoxia would be greater than in normal pregnancies with a consequent higher production of VEGF and sFLT-1 and lower PLGF. However, two studies that measured both VEGF and sFLT-1 reported that in pregnancies that subsequently developed preeclampsia first-trimester serum sFLT-1 was not significantly different from normotensive controls;[22,23] in one study serum VEGF was increased in the group that developed preeclampsia,[23] and the other study reported that in the majority of cases of preeclampsia and controls the concentrations of VEGF were undetectable.[22] We postulate that in the presence of impaired, rather than normal, placentation early placental hypoxia and increased production of VEGF is not accompanied by increase in sFLT-1 because of the inability of the impaired placenta to produce this receptor; alternatively there is a limited ability of the impaired placenta to produce both VEGF and sFLT-1.

Clinical implications

One fifth of women with chronic hypertension develop superimposed preeclampsia; ideally first-trimester biomarkers could help distinguish between women with chronic hypertension that would develop preeclampsia from those that would not so that appropriate pharmacological interventions and surveillance would be undertaken. Although we found that first-trimester serum PLGF and sFLT-1 were lower in women with chronic hypertension that developed superimposed preeclampsia, compared to

those that did not, there was a substantial overlap in values between the groups and it is therefore unlikely that these biomarkers will be useful in stratifying care of women with chronic hypertension.

These findings have significant clinical and research implications regarding the development of prediction models and prophylactic pharmacological strategies for preeclampsia. It remains uncertain as to what prophylactic pharmacological measures may help in reducing the risk of preeclampsia in women with chronic hypertension. In women at high-risk of preeclampsia prophylactic use of low-dose aspirin starting from the first trimester reduces the risk of preterm preeclampsia and length of stay in the neonatal intensive care unit by >60%.[52-54] However, the beneficial effect of aspirin in the prevention of preterm preeclampsia may not apply in pregnancies with chronic hypertension.[52] It is likely that aspirin mediates its' effect in women at high risk of preeclampsia at least, in part, through inhibiting the trophoblastic production of sFLT-1 and, thereby, increasing PLGF.[55] However, it has been shown that aspirin has no effect on the sFLT-1/PLGF ratio in women with chronic hypertension[55] where our findings have demonstrated lower first trimester sFLT-1. Therefore, aspirin is not able to exert as significant an effect on improving the degree of placental impairment when compared to those without chronic hypertension. It is possible that in women with chronic hypertension, pre-existing endothelial dysfunction may be the primary cause and placental disease the aftermath. Further research should be focused on the interplay between maternal cardiovascular system, endothelial and placental function.

Strengths and limitations

The strengths of this study are examination of a large population of women with chronic hypertension recruited in the first trimester of pregnancy and followed up with a standardized policy for strict control of BP throughout pregnancy. Furthermore, the exclusion of women with underlying renal or liver disease at booking has reduced the heterogeneity of the population, thereby, avoiding bias in the diagnosis of preeclampsia.

A limitation of the study is that we have not measured VEGF concentrations to prove our hypothesis of low sFLT-1 due to lack of VEGF up-regulation; unfortunately current assays for VEGF are of limited reliability.[22,23] Additionally, we have not measured sFLT-1 and PLGF concentrations outside pregnancy to correlate values with possible changes in pregnancy and outcome.

Conclusion

Women with chronic hypertension, and particularly those who subsequently developed preeclampsia, have reduced first trimester concentrations of both PLGF and sFLT-1, but it is unlikely that these biomarkers will be useful in stratifying pregnancy care of women with chronic hypertension.

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Table 1: Comparison of maternal and pregnancy characteristics between normotensive controls, women with chronic hypertension who did not develop superimposed preeclampsia and those who did.

Variable	Controls (N=142)	Chronic hypertension		Overall p-value
		No preeclampsia (n=448)	Preeclampsia (n=202)	
Characteristics at 11-13 weeks				
Age (years), median (IQR)	31.5 (28.2-34.5) §†	34.0 (31.0-38.0)	34.0 (31.0-38.0)	<0.0001
Age (years), median (IQR)	31.5 (28.2-34.5) §†	34.0 (31.0-38.0)	34.0 (31.0-38.0)	<0.0001
Weight (kg), median (IQR)	81.9 (71.7-90.0)	84.5 (71.6-97.0)	83.0 (69.9-97.0)	NS
Height (meters), median (IQR)	165.0 (160.0-169.6)	165.0 (161.0-170.0)	165.0 (160.0-168.3)	NS
Body mass index (kg/m^2), median (IQR)	30.1 (26.6-33.5)	31.0 (26.0-36.0)	31.0 (26.0-35.6)	NS
Gestational age, median (IQR)		10.0 (9.3-11.1)	10.0 (9.1-11.3)	NS
Racial origin				
Black, n (%)	87 (61.3)	257 (57.4)	132 (65.3)	NS
White, n (%)	52 (36.6)	150 (33.5)	59 (29.2)	NS
Other, n (%)	3 (2.2) §†	41 (9.2)†	11 (5.4)	0.011
Parous, n (%)	102 (71.8)	311 (69.4)	135 (66.8)	NS
Previous preeclampsia, n (%)	5.0 (3.5) §†	163 (36.4)	92 (45.5) ‡	<0.0001
Family history of preeclampsia, n (%)	7.0 (4.9) §†	51 (11.4)	35 (17.3) ‡	0.002
Systolic BP (mmHg), median (IQR)	118.1 (112.9-125.0) §†	130.0 (120.0-140.0)	130.0 (120.0-140.0)	<0.0001
Diastolic BP in (mmHg), median (IQR)	73.0 (66.0-77.1) §†	80.0 (74.0-88.0)	80.0 (75.0-90.0)	<0.0001
Antihypertensive medications, n (%)		228 (50.9)	142 (70.2) ‡	<0.0001
Pregnancy outcome				
Antihypertensive medications at delivery, n (%)		227 (50.7)	181 (89.6) ‡	<0.0001
Severe hypertension, n (%)		68 (15.2)	86 (42.6) ‡	<0.0001
Preterm preeclampsia, n (%)		-	119 (58.9)	-
Gestation at delivery, median (IQR)	40.2 (39.4-40.9) §†	39.1 (38.4-40.1)	37.7 (35.6-38.9) ‡	<0.0001
Birth weight percentile, median (IQR)	45.6 (30.2-69.0) †	42.3 (21.7-69.7)	13.9 (1.2-52.7) ‡	<0.0001
Birthweight <10th centile, n (%)	2 (1.4) §†	52 (11.6)	92 (45.5) ‡	<0.0001

IQR=interquartile range

§ Statistically significant difference between the controls and the group of chronic hypertension that did not develop preeclampsia

† Statistically significant difference between the controls and the group of chronic hypertension that developed preeclampsia

‡ Statistically significant difference in the chronic hypertension group between those that developed preeclampsia and those that did not.

Table 2: Comparison of maternal serum placental growth factor (PLGF) and soluble fms-like tyrosine kinase-1 (sFLT-1) and the sFLT-1 / PLGF ration between normotensive controls, women with chronic hypertension who did not develop superimposed preeclampsia and those who did.

Biomarkers	Controls (n=142)	Chronic hypertension		Overall p-value
		No preeclampsia (n=448)	Preeclampsia (n=202)	
PLGF MoM, median (IQR)	1.009 (0.901-1.111) §†	0.948 (0.814-1.093)	0.904 (0.771-1.052) ‡	<0.0001
sFLT-1 MoM, median (IQR)	0.991 (0.905-1,108) §†	0.938 (0.807-1.095)	0.895 (0.760-1.033) ‡	<0.0001
sFLT-1/PLGF ratio MoM, median	1.009 (0.861-1.159)	1.040 (0.885-1.241)	1.050 (0.892-1.281)	NS

IQR=interquartile range

§ Statistically significant difference between the controls and the group of chronic hypertension that did not develop preeclampsia
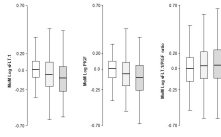
† Statistically significant difference between the controls and the group of chronic hypertension that developed preeclampsia

‡ Statistically significant difference in the chronic hypertension group between those that developed preeclampsia and those that did not.
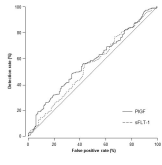
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FIGURE LEGEND

Figure 1: Serum concentrations of multiples of the median (MoM) Log soluble fms-like tyrosine kinase-1 (sFLT-1), MoM Log placental growth factor (PLGF) and MoM Log sFLT-1/PLGF ratio in controls (white box), women with chronic hypertension who did not develop preeclampsia (light grey box) and women with chronic hypertension who developed preeclampsia (dark grey box).

Figure 2: Receiver operating characteristic curves for the prediction of superimposed preeclampsia by multiples of the median log soluble fms-like tyrosine kinase-1 (interrupted line) and MoM log placental growth factor (solid line).