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Angiogenic profile and smoking in the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) cohort

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Running title: Pre-eclampsia and smoking

ABSTRACT

Objectives The biological mechanism by which smoking reduces the risk of pre-eclampsia (PE) is unresolved. We studied first and third trimester serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF) and soluble endoglin (sEng) to ascertain whether these factors are altered in mothers who smoke.

Subjects and Methods First trimester serum samples were available from 217 women who later developed PE and 238 control women who did not develop PE. Third trimester serum samples were available from 174 PE and 54 non-PE women.

Results PE women who smoked during pregnancy had elevated first trimester concentrations of serum PlGF [geometric mean (95% CI): 39.8 (32.6-48.5) pg/ml, $p=0.001$] and reduced sEng concentration [5.0 (4.6-5.6) ng/ml, $p=0.047$] compared to PE non-smokers [30.0 (28.1-32.1) pg/ml and 6.1 (5.9-6.4) ng/ml, respectively]. Similar differences were observed among controls. For PlGF, a dose-response effect was suggested among smoking PE women.

Conclusion We provide evidence that the protective effect of smoking in reducing the risk of PE may be due to the early pregnancy change towards angiogenic marker profile. Also, in late pregnancy, smoking exerted an overall angiogenic effect in PE pregnancies, and may complicate its use as a prognostic and diagnostic marker.

Keywords: angiogenic markers, pre-eclampsia, pregnancy, smoking

Key messages:

- Smoking appears to have angiogenic effects in early pregnancy with reduced sFlt-1 and sEng concentrations and elevated PlGF concentrations in both normal and PE pregnancies.
- In late pregnancy, smoking seems to exert an overall angiogenic effect in PE pregnancies, and may complicate its use as a prognostic and diagnostic marker.

Introduction

Pre-eclampsia (PE) is a complex pregnancy disorder defined by new-onset hypertension and proteinuria after 20 weeks of gestation, or new-onset PE-associated signs in the absence of proteinuria (1). The pathogenesis of PE remains poorly understood due to its heterogeneous, multi-systemic nature. The conventional risk factors for the development of PE include nulliparity, multifetal gestations, previous history of PE, chronic hypertension, obesity, diabetes mellitus, vascular and connective tissue disorders, age >35 years at first pregnancy and African-American ethnicity (2). An obstetrical paradox is that maternal smoking appears to be protective for the development of PE. There is a robust body of literature that has consistently demonstrated a reduced risk of PE in women who smoke when they are pregnant (3-5). However, the biological mechanism by which smoking during pregnancy reduces the risk of PE is still unresolved.

In the last decade, research attention has increasingly focused on imbalances in maternal pro-angiogenic placental growth factor (PlGF) and anti-angiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) in the pathogenesis of PE (6-10). Elevated levels of s-Flt1 and reduced levels of PlGF have been observed in patients with PE, as well as in pregnant women prior to development of PE (6). The high sFlt-1/PlGF ratio has been demonstrated to already exist before PE is established, and thus might serve as a diagnostic tool to predict future PE. Different cut-offs for the sFlt1/PlGF ratio have also been proposed to allow for the assessment of PE (10-13). The soluble form of endoglin (sEng) has also been shown to have anti-angiogenic activity and increased levels have been reported in PE women two to three months before the onset of PE (7).

Different concentrations of angiogenic markers have been reported in smoking and non-smoking pre-eclamptic women. Smoking appears to cause a pro-angiogenic state, particularly by lowering

maternal sFlt-1 concentrations (14-16). The effect of smoking on PlGF and sEng concentrations is less well defined.

In this observational study, we have compared the first and third trimester serum concentrations of sFlt-1, PlGF and their ratio, in addition to sEng, in women who had smoked before or during pregnancy to those who had never smoked. Since altered levels of these markers precede the clinical onset of the disease, the PE and non-PE (control) mothers were investigated separately. We also tested within these subgroups recently recommended rule-out cut-off values.

Methods

Study design and aim

Data for the present study comes from the Finnish Genetics of Pre-eclampsia Consortium (FINNPEC), a cross-sectional case-control multicentre study with a nationwide clinical and DNA database on PE and non-PE (control) women, including their partners and infants, established in order to identify genetic risk factors for PE. Details of the study design, methods and procedures have been described elsewhere (17). Herein, we investigated whether maternal serum concentrations of sFlt-1, PlGF, sEng and sFlt-1/PlGF ratio available from FINNPEC participants associate with maternal smoking status and number of cigarettes smoked.

Study subjects

Originally in the FINNPEC Study, 1450 patients with PE and 1065 control women without PE were recruited at the five Finnish university hospitals. In this study, we focused on a subset of women from whom first and third trimester serum samples were available. All participants provided written

informed consent, and the FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa.

Inclusion criteria

Nulliparous or multiparous women with a singleton pregnancy were eligible for the study. PE was defined as hypertension and proteinuria occurring after 20 weeks of gestation. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, and proteinuria as the urinary excretion of ≥ 0.3 g protein in a 24-hour specimen, or 0.3g/l, or two $\geq 1+$ dipstick readings in a random urine determination with no evidence of a urinary tract infection. Women who suffered from proteinuria without hypertension (n=5) were included in the control group. Furthermore, women who suffered from gestational hypertension but did not meet the full criteria for PE were included in the control group. Each diagnosis was ascertained from hospital records and independently confirmed by a research nurse and a study physician.

Exclusion criteria

Exclusion criteria were multiple pregnancy, maternal age less than 18 years and an inability to provide informed consent based on information being offered in Finnish or Swedish.

Background and obstetric data

Extensive information on family history, medical history, obstetric history, pregnancy complications, pregnancy outcome, proteinuria, blood pressure, laboratory measurements, delivery and newborn was obtained from the hospital records and maternity cards (women in Finland are given a personal maternity card at their first visit to a maternity centre, and all visits are recorded on these cards). Data on pre-pregnancy weight, height, and diastolic and systolic blood pressure at first antenatal visit were obtained from maternity cards. Data on smoking status and the number of cigarettes smoked were

collected from the maternity cards and complemented from the background information questionnaires if needed. The mother was considered to be a pre-pregnancy smoker if she smoked within 6 months of conception. Mothers were further divided into subgroups based on their smoking status (current smokers/pre-pregnancy smokers/non-smokers).

Serum samples and angiogenic markers

First and third trimester serum samples were collected from women receiving care from the Hospital District of Helsinki and Uusimaa. First trimester serum samples were obtained via first trimester biochemical screening for fetal chromosome abnormalities (range 9-15 weeks of gestation), and serum samples from the second and third trimesters (range 20-42 weeks of gestation) were collected at hospitals for study protocol.

Maternal serum sFlt-1 and PlGF concentrations were measured using sFlt-1 and PlGF electrochemiluminescence immunoassays (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany) on a cobas e601 analyser (Hitachi High Technology Co, Tokyo, Japan). Serum concentrations of sEng (CD105) were measured using the human Quantikine Endoglin ELISA kit (R&D Systems, UK) according to the manufacturer's instructions.

Recently recommended rule-out cut-off values of 33 (20 weeks to delivery), rule-in cut-offs of 85 (until 33 weeks 6 days) and 110 (34 weeks to delivery) were tested for the Elecsys immunoassay sFlt-1/PlGF ratio (NICE 2016). Furthermore, we tested a cut-off for the sFlt-1/PlGF ratio that was presented very recently in the PROGNOSIS study (10). Zeisler et al. (10) derived a single cut-off value independent of the weeks gestation: values below 38 were considered negative and were used to rule-out PE within 1 week after assessment of the ratio.

Statistical analysis

Statistical tests were performed with IBM SPSS Statistics version 20 (Armonk, NY, USA). The normality of variable distributions was verified with the Kolmogorov–Smirnov test. Logarithmic transformation was used when appropriate. Each biomarker was ln-transformed to correct for right-skewness, and estimated means were back-transformed as geometric means and 95% confidence intervals for purposes of presentation. For the continuous variables, comparisons between groups were made with general linear model (GLM) univariate analyses with adjustments. For the categorical variables, the comparisons were performed with the chi-squared test and logistic regression. With skewed distributions, comparisons between continuous variables were performed by the Mann Whitney U-test.

Results

The maternal and perinatal characteristics of PE and control women according to smoking status are presented in Table I. Smokers were younger in both PE and control groups and, in the PE group, smokers had higher body mass index (BMI) compared to non-smokers. There were no differences in any other characteristics between smokers and non-smokers including birthweight, gestational weeks and percentage of small for gestational age (SGA) fetuses (birthweights below -2.0 SD units, according to Finnish standards).

Concentrations of angiogenic markers in PE and control women according to smoking status are presented in Table II. Figure 1 represents the relative levels of sFlt-1, PlGF and sEng compared with the non-smoking, non-PE referent group. There were no differences in sFlt-1 or sEng concentrations at early pregnancy (first trimester) between PE and control non-smokers. In late pregnancy, PE women had elevated concentrations of both sFlt-1 (+170.5%) and sEng (+149.1%) compared to non-smoking controls (Figure 1B and 1F). PE non-smoking women also had reduced levels of PlGF at

first and third trimester time points (-27.0% and -54.4%, respectively) (Figure 1C and 1D). Furthermore, the sFlt-1/PlGF ratio was elevated (+347.6%) in the PE group compared to controls (Figure 1G).

There were no differences in sFlt-1 or sEng concentrations in early non-PE pregnancies between the current, pre-pregnancy smokers and the non-smoking comparison group (Table II and Figure 1), whereas smoking control women had 35.5% higher PlGF concentrations. In late pregnancies, a tendency for reduced PlGF (23.3%) was found among smoking compared to non-smoking, unaffected women, whereas sFlt-1 and sEng did not differ between these groups.

Smoking and PE both affected sFlt-1 in the same direction in early pregnancy, both tending to reduce the concentration with the net effect resulting in a 20.5% reduction. For PlGF, the effects of smoking and PE in early pregnancy were in the opposite directions compared to previous, implying that the concentrations of sFlt-1 and PlGF came close to the non-smoking, unaffected comparison group. In late pregnancy, PlGF concentrations were lower for both smoking and PE as single exposures, but their net effect in smoking PE women was not additive, with an overall 45.9% reduction. Accordingly, elevations of sFlt-1 and sEng during late pregnancy (97.4-128.9% and 113.9-130.1%, respectively) by concomitant smoking and PE were smaller than the changes caused by PE without smoking, when compared to the non-smoking comparison group.

PE women who smoked during pregnancy had elevated first trimester concentrations of PlGF and reduced sEng concentrations, compared to PE non-smokers (Table II). Similar differences were observed among controls. In addition, the proportion of PE women whose sFlt-1/PlGF ratio was below the cut-off of 33 (rule-out according to the NICE guideline) was higher in the group of smoking mothers. There were no differences in the percentages of smoking and non-smoking women

exceeding the cut-offs of 85 (rule-in between 20 and 33 weeks 6 days according to the NICE guideline) or 110 (rule-in between 34 weeks 0 days and delivery according to the NICE guideline) when the sampling time was taken into account. In the controls, there were women who exceeded both NICE rule-in cut-offs (85 and 110) (Table II), but there were no differences in the proportions of women below or above the cut-offs between smokers and non-smokers in the control group.

Angiogenic markers and number of cigarettes smoked before pregnancy

The first trimester concentrations of sFlt-1, PlGF, sEng and the sFlt-1/PlGF ratio in pre-eclamptic and control groups according to the number of cigarettes smoked daily before pregnancy are presented in Supplementary Figure 1. An association was found between the number of cigarettes smoked and concentrations of PlGF in pre-eclamptic women. Those PE women who smoked 16 or more cigarettes per day had higher concentrations of PlGF than non-smoker PE women (Supplementary Figure 1B). Furthermore, an association was found between the number of cigarettes smoked and the sFlt-1/PlGF ratio in both PE and control women (Supplementary Figure 1C). In PE women, the more cigarettes smoked, the lower the sFlt1-1/PlGF ratio. Whereas, in controls, the opposite association was observed. There was no association between sFlt-1 concentration and the number of cigarettes smoked (Supplementary Figure 1A), but among PE women, a higher number of cigarettes was associated with lower concentrations of sEng (Supplementary Figure 1D).

Angiogenic markers and number of cigarettes smoked during pregnancy

The first trimester concentrations of sFlt-1, PlGF, sEng and the sFlt1/PlGF ratio in pre-eclamptic and control groups according to the number of cigarettes smoked during pregnancy are presented in Supplementary Figure 2. An association was found between the number of cigarettes smoked and concentrations of PlGF in both pre-eclamptic and control women (Supplementary Figure 2B): the more cigarettes smoked, the higher the PlGF concentration. In addition, an association was found

between the number of cigarettes smoked and the sFlt-1/PlGF ratio in both PE and control women (Supplementary Figure 2C): the more cigarettes smoked, the lower the sFlt1-1/PlGF ratio. An association between sFlt-1 and sEng and the number of cigarettes smoked was observed among controls only (Supplementary Figures 2A and 2D).

Discussion

We studied the first and third trimester serum concentrations of sFlt-1, PlGF and their ratio, in addition to sEng, in PE and control women according to their smoking status in the FINNPEC cohort. We had several interesting findings. First, the early pregnancy effects of smoking and PE on PlGF concentrations were on the order of 27-35%, but in opposite directions, thus, smoking appears to normalise the PlGF reduction in women at risk for developing PE. With sFlt-1 and sEng, all changes were on the order of 20% or less and they were not significant. Second, the scale of late pregnancy differences for all markers was very different from early pregnancy, with changes on the order of 50-150%. Smoking in unaffected pregnancies and PE in non-smoking women both tended to reduce PlGF up to 50%, but the reduction was similar in smoking PE women, showing that the effect of the two concomitant exposures was not additive. Similarly, the differences in sFlt1 and sEng associated with smoking were non-significant when compared to the 150% elevation in the marker levels due to PE, but they tended to normalise changes brought about by PE. Third, different late pregnancy cut-off values to predict or diagnose PE tended to be less sensitive among smoking women. Finally, women who were pre-pregnancy smokers also showed differences in marker levels which collectively were in between smokers and non-smokers.

The overall interpretation of the results is that smoking appeared to have angiogenic effects in early pregnancy with reduced sFlt-1 and sEng concentrations and elevated PlGF concentrations in both normal and PE pregnancies. Although differences in PlGF concentrations were only significant

between smokers and non-smokers, a similar angiogenic trend in sFlt1 and sEng concentrations in pre-pregnancy smoking women supports this conclusion. In addition, pooling pre-pregnancy and current smokers made the sEng concentration, but not sFlt-1, differences significant (data not shown). Within sEng, some dose-response effect was also suggested. Serum concentrations of sFlt-1, sEng and sFlt-1/PlGF ratio levels are usually found to be higher and PlGF concentrations lower and, thus, anti-angiogenic in PE (6, 10, 18). The obstetrical paradox that maternal smoking appears to be protective for the development of PE is in line with the present results. On the other hand, timing is a key question because Khalil et al. (19) have shown that maternal sPlGF is a useful marker for PE from the first trimester onward, while the level of sFlt-1 is likely to have a predictive value from the second trimester onward. For sEng, there is some evidence that the elevation precedes the clinical onset of the disease and may already be apparent from the first trimester of pregnancy (20).

Interestingly, in pre-eclamptic women, smoking was rather angiogenic than anti-angiogenic in late pregnancy. Smoking in late pregnancy tended to exert an anti-angiogenic effect via reduced PlGF levels in non-PE women, whereas the effects on sFlt-1 and sEng production were virtually non-existent. It should be noted, however, that the number of current smokers was very low in the control group and the inter-individual variations in serum concentrations were large.

Overall, PE and pregnancy are each themselves such strong contributors to angiogenic levels in late pregnancy that the effect of smoking is very moderate. While the sFlt1/PlGF ratio among PE women in the three groups during late pregnancy was higher compared to non-smoking controls, the ratio is lowest and, thus, the net angiogenic effect highest in the PE group of current smokers. This will have straightforward implications for clinical decision making in antenatal care. There is an obvious need to adjust clinical risk algorithms according to smoking status.

Although there are no formal guidelines regarding the use of the sFlt-1/PlGF ratio, consensus statements have been developed by international experts on the clinical use of the Elecsys immunoassay sFlt-1/PlGF ratio. We retrospectively investigated the feasibility of these relatively recently established sFlt-1/PlGF cut-offs according to smoking status. The proportion of PE women whose ratio was below the cut-off of 33 was higher in the group of PE mothers who smoked, when the sampling time was taken into account. This may suggest that the utility of a cut-off point of 33 (20 weeks to delivery) may differ between smokers and non-smokers. However, larger studies are needed to confirm these observations.

Strengths and limitations

Our study has several strengths. The diagnostic criteria for PE were well defined. Furthermore, detailed clinical information allowed us to accurately define the phenotypes. Detailed phenotyping enabled us to include various adjustments for maternal characteristics. Clinical covariates affecting angiogenic levels (parity, age, BMI, gestational weeks at sampling) were taken into account when aiming to define the role of smoking in PE. Furthermore, data on smoking were collected from two distinct sources which could reduce the possible bias in maternal report of smoking.

Our study also has certain limitations. The inter-individual variations in serum concentrations were relatively large and the sample size was small, especially when further dividing into subcategories. In addition, there was a very small number of samples available from the second trimester. We also lack biochemical confirmation of smoking status (e.g., cotinine concentrations). It has been postulated that products other than the nicotine in cigarettes might protect against PE (5). Wikström et al. (5) assessed the effect of snuff use during pregnancy and it was not observed to reduce the risk for PE. This suggests that exposure to the combustible products of cigarette smoke, such as carbon monoxide may offer the protective effect for PE. This study, however, did not address the biological mechanisms

by which smoking may affect angiogenic factors in PE women, and further studies are warranted to define the pathways.

Conclusion

We provide further evidence that the protective effect of smoking in reducing the risk of PE may be due to the early pregnancy change towards an angiogenic marker profile. Also in late pregnancy, smoking seems to exert an overall angiogenic effect in PE pregnancies, and may complicate its use as a prognostic and diagnostic marker.

Disclosure of interest

The authors state that there are no conflicts of interest. Funding was received from the Jane and Aatos Erkko Foundation, Päivikki and Sakari Sohlberg Foundation, Academy of Finland (grants 121196, 134957, and 278941), Research Funds of the University of Helsinki, government special state subsidy for health sciences (In Finnish; Erityisvaltionosuus) at the Hospital District of Helsinki and Uusimaa, Finnish Medical Foundation, Finska Läkaresällskapet, Novo Nordisk Foundation, Finnish Foundation for Pediatric Research, Emil Aaltonen Foundation, and Sigrid Jusélius Foundation.

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Figure legends:

Figure 1. Relative levels of sFlt-1, PlGF, sEng at early and late pregnancy and sFlt-1/PlGF ratio at late pregnancy, compared with non-smoking controls. * $p < 0.05$ and ** $p \leq 0.001$ Bonferroni-adjusted comparisons with non-smoking controls. (Numbers below the bars represent the number of subjects in each category. Note that scale differs between the figures.)

Supplementary Figure 1. Ln-transformed first trimester concentrations of sFlt-1 (A), PlGF (B), sFlt1/PlGF ratio (C) and sEng (D) in pre-eclamptic and control groups according to smoking status and the number of cigarettes smoked before pregnancy.

Supplementary Figure 2. Ln-transformed first trimester concentrations of sFlt-1 (A), PlGF (B), sFlt1/PlGF ratio (C) and sEng (D) in pre-eclamptic and control groups according to smoking status and the number of cigarettes smoked during pregnancy.

Table I. Maternal and perinatal characteristics of pre-eclamptic and control groups, according to smoking status in FINNPEC.

	PRE-ECLAMPSIA					CONTROL			
	Current smokers (n=29)	Pre-pregnancy smokers (n=50)	Non-smokers (n=228)	<i>p</i>		Current smokers (n=24)	Pre-pregnancy smokers (n=45)	Non-smokers (n=197)	<i>p</i>
Age at delivery (years)	28.8 ± 8.3	28.2 ± 4.9	31.7 ± 5.0	<0.001*		27.9 ± 5.8	28.9 ± 5.2	31.4 ± 4.5	<0.001*
Nulliparous, n (%)	20 (69.0%)	37 (74.0%)	172 (75.4%)	0.748		18 (75.0%)	13 (28.9%)	107 (54.3%)	0.029
BMI, kg/m ² (self-reported, pre-pregnancy) ^h	27.6 ± 6.8	26.7 ± 6.3	24.8 ± 4.9	0.003 ^b		25.4 ± 5.1	24.0 ± 4.1	23.7 ± 3.7	0.055 ^b
PAPP-A (mU/l)	1137 ± 921 (26)	1320 ± 946 (41)	1537 ± 1254 (204)	0.097 ^a		1650 ± 1286 (21)	2010 ± 1379 (44)	1989 ± 1158 (187)	0.275 ^a
Beta hCG (ug/l)	47 ± 39 (26)	39 ± 20 (41)	48 ± 31 (204)	0.286 ^a		57 ± 63 (21)	53 ± 42 (44)	54 ± 36 (187)	0.958 ^a
Highest systolic blood pressure (mm Hg) ^g	165 ± 16	164 ± 17	167 ± 17	0.825 ^c		133 ± 12	129 ± 16	127 ± 16	0.341 ^c
Highest diastolic blood pressure (mm Hg)	107 ± 8	111 ± 9	111 ± 9	0.151 ^c		87 ± 10	86 ± 9	86 ± 11	0.939 ^c
Proteinuria (max.) (g/24 h)	3.7 ± 3.4 (28)	3.5 ± 3.2 (49)	4.1 ± 3.4 (218)	0.118		-	-	-	
Chronic hypertension ^e	1 (3.4%)	9 (18.0%)	42 (18.4%)	0.126		2 (8.3%)	3 (6.7%)	7 (3.6%)	0.423
Gestational hypertension ^f	-	-	-			3 (12.5%)	3 (6.7%)	21 (10.7%)	0.670
Gestational diabetes mellitus	6 (20.7%)	6 (12.0%)	25 (11.0%)	0.317		3 (12.5%)	3 (6.7%)	8 (4.1%)	0.195
Pre-gestational diabetes mellitus									
Type 1 diabetes	3 (10.3%)	2 (4.0%)	9 (3.9%)	0.292		0	0	1 (0.5%)	-
Type 2 diabetes	0	0	1 (0.4%)	-		0	0	0	-
Mode of delivery									
Vaginal	20 (69.0%)	24 (48.0%)	121 (53.0%)			18 (75.0%)	33 (73.3%)	161 (82.1%) (196)	
Caesarian section	9 (31.0%)	26 (52.0%)	107 (47.0%)	0.182		6 (25.0%)	12 (26.7%)	35 (17.9%) (196)	0.335
Gestational weeks at delivery	36.6 ± 2.5	36.7 ± 3.3	36.4 ± 3.4	0.924 ^b		38.8 ± 3.5	40.0 ± 1.9	39.1 ± 2.8	0.134 ^b
Birthweight, g	2834 ± 743	2846 ± 884	2683 ± 883	0.158 ^d		3315 ± 930	3591 ± 573	3421 ± 769	0.886 ^d

Relative birthweight (SD)	-0.75 ± 1.25	-0.72 ± 1.42	-1.06 ± 1.29	0.116 ^d	-0.40 ± 1.43	-0.04 ± 0.94	-0.22 ± 1.2	0.755 ^d
Fetal sex, female/male (%)	17/12 (58.6%/41.4%)	26/24 (52.0%/48.0%)	120/108 (52.6%/47.4%)	0.819	9/15 (37.5%/62.5%)	18/27 (40.0%/60.0%)	92/105 (46.7%/53.3%)	0.542
SGA	4 (13.8%)	9 (18.0%)	47 (20.6%)	0.653	3 (12.5%)	2 (4.4%)	19 (9.6%)	0.450

BMI=body mass index, PAPP-A=placental protein A, β-hCG=beta human chorionic gonadotrophin, SGA=small-for-gestational age, SD=standard deviation

()number of available information/samples unless from all

Data are presented as mean ± SD or percentages

*non-parametric tests used

^aadjusted for gestational weeks at sampling

^badjusted for parity, mother's age at birth

^cadjusted for parity, mother's age at birth, pre-pregnancy BMI

^dadjusted for parity, mother's age at birth, gestation weeks, pre-pregnancy BMI, chronic hypertension, gestational diabetes, pre-gestational diabetes mellitus

^esystolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg detected before 20 weeks of gestation

^fblood pressure ≥140/90, no proteinuria

^gwhen highest diastolic value recorded

^hbased on weight and height before pregnancy, self-reported at first antenatal visit

Table II. Concentrations of angiogenic markers in pre-eclamptic and control women according to smoking status, geometric mean (95% CI).

	PRE-ECLAMPSIA				CONTROL			
	Current smokers (n=29)	Pre-pregnancy smokers (n=50)	Non-smokers (n=228)	<i>p</i> *	Current smokers (n=24)	Pre-pregnancy smokers (n=45)	Non-smokers (n=197)	<i>p</i> *
sFlt-1 (pg/ml)								
I trimester	1107.7 (960.1-1286.9) (24)	1224.1 (1053.6-1422.3) (34)	1352.9 (1261.4-1447.9) (159)	0.499	1281.7 (1064.2-1537.6) (20)	1418.0 (1268.9-1584.8) (37)	1394.1 (1312.9-1475.9) (181)	0.684
II-III trimester	9395.6 (6558.4-13467.0) (13)	8103.1 (5710.2-11498.8) (31)	11103.3 (10097.1-12209.9) (130)	0.286	5934.9 (3420.4-10298.0) (4)	4536.9 (3190.7-6457.5) (13)	4105.2 (3377.9-5004.0) (36)	0.361
PlGF (pg/ml)								
I trimester	39.8 (32.6-48.5) (24) ^c	32.6 (27.8-38.2) (34)	30.0 (28.1-32.1) (158)	0.001	55.7 (48.5-64.4) (20) ^{c,d}	38.5 (34.0-43.7) (37)	41.1 (38.8-43.5) (181)	<0.001
II-III trimester	93.6 (69.3-126.4) (13)	83.9 (61.0-116.3) (31)	79.0 (71.0-87.9) (130)	0.357	132.8.0 (48.2-366.1) (4)	184.9 (131.2-261.1) (13)	173.1 (126.6-236.8) (36)	0.655
sEng (ng/ml)								
I trimester	5.0 (4.6-5.6) (24) ^c	5.6 (5.0-6.2) (34)	6.1 (5.9-6.4) (158)	0.043	4.7 (4.3-5.1) (20) ^c	5.6 (5.1-6.1) (37)	5.7 (5.5-6.0) (181)	0.013
II-III trimester	39.8 (24.8-64.1) (13)	37.0 (26.8-50.9) (31)	43.1 (38.5-47.9) (130)	0.837	13.7 (3.3-57.2) (4)	14.5 (10.0-21.2) (13)	17.3 (14.1-21.1) (36)	0.871
sFlt-1/PlGF ratio								
II-III trimester	148 ± 170 (13) ^a	209 ± 275 (31) ^a	188 ± 152 (130) ^a	0.249	64 ± 70 (4) ^a	41 ± 48 (13) ^a	42 ± 44 (36) ^a	0.362
sFlt-1/PlGF ratio <33, n (%) (20+0-delivery)	1 (7.7%) (13)	6 (19.4%) (31)	3 (2.3%) (130)	0.001^b	3 (75.0%) (4)	9 (69.2%) (13)	20 (55.6%) (36)	0.567 ^b
sFlt-1/PlGF ratio <38, n (%) (24+0-36+6 gwk)	1 (10.0%) (10)	4 (21.1%) (19)	2 (3.2%) (63)	0.396 ^b	0 (0.0%) (1)	-	3 (37.5%) (8)	0.444 ^b
sFlt-1/PlGF ratio ≥85, n (%) (20+0-33+6 gwk)	4 (66.7%) (6)	6 (85.7%) (7)	30 (85.7%) (35)	0.504 ^b	1 (100.0%) (1)	-	1 (16.7%) (6)	0.286 ^b
sFlt-1/PlGF ratio ≥110, n (%) (34+0-delivery)	2 (28.6%) (7)	15 (62.5%) (24)	54 (56.8%) (95)	0.276 ^b	0 (0.0%) (3)	2 (15.4%) (13)	2 (6.7%) (30)	0.556 ^b

CI= confidence interval; sFlt-1= soluble fms-like tyrosine kinase 1; PlGF= placental growth factor, sEng=soluble endoglin

^aadjusted for parity, pre-pregnancy BMI, mother's age at birth, gestational weeks at sampling^aarithmetic mean ± SD^bχ² test^ccurrent vs. non-smokers, *p*<0.05^dcurrent vs. pre-pregnancy smokers, *p*<0.05