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ORIGINAL ARTICLE

Additive effects of mean blood pressure and bilateral notching in the second trimester on subsequent angiogenesis-related factors

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It has not been clarified whether high mean blood pressure (HBP) of ⩾90 mm Hg and bilateral notching (BN) on uterine artery Doppler additively affect the subsequent circulating levels of placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng). Serum levels of PlGF, sFlt-1 and sEng at 17–25 weeks and 26–32 weeks were measured in all women with HBP + BN − (*n* = 272), HBP − BN + (*n* = 130) and HBP + BN + (*n* = 60) in 1239 eligible women, and 338 consecutive women with HBP − BN − were selected from the remaining 777 women. Only data before the onset of preeclampsia were evaluated. The cutoff value of an abnormal decrease of PlGF was set at the 5th percentile, and those of an abnormal increase of sFlt-1, sFlt-1/PlGF and sEng were set at the 95th percentile. The frequency of HBP in those with BN − was almost the same as that in those with BN + (25.9% *vs.* 26.7%). In women with HBP − BN −, HBP − BN +, HBP + BN − and HBP + BN +, the frequency of abnormal sFlt-1/PlGF ratio at 26–32 weeks was 6.6%, 9.2%, 14.4% and 22.8%, respectively; and the frequency of abnormal sFlt-1/PlGF ratio at 26–32 weeks in those with HBP + BN + was significantly increased than in HBP − BN −. Similarly, in the four groups, the frequency of abnormal sEng at 26–32 weeks was 5.4%, 2.5%, 12.2% and 19.0%, respectively; and the frequency in those with HBP + BN + was significantly increased than in HBP − BN −. In conclusion, high BP levels and abnormal uterine artery Doppler may be additively implicated in circulating abnormalities of angiogenesis-related factors.
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Keywords: bilateral notching; blood pressure levels; placental growth factor; soluble endoglin; soluble fms-like tyrosine kinase 1

INTRODUCTION

High blood pressure (HBP) and chronic hypertension are predictors of the occurrence of preeclampsia (PE).[1–3] Bilateral notching (BN), high resistance index, high pulsatility index (PI) or high notch depth index on the uterine artery Doppler (UAD) in the first and second trimesters are also predictors of PE.[4–7] Both HBP and abnormal UAD in the second trimester were independent risk factors for PE,[8–10] even in the first trimester.[11] Abnormal increases in soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), accompanied by the decrease of placental growth factor (PlGF), are also implicated in the generation of PE.[12–17] These increases are believed to be produced by placental hypoxia because of reduced perfusion, clinically represented as abnormal UAD.[14] We showed that HBP, BN, plasma levels of the sFlt-1/PlGF ratio and plasma levels of hydroxysteroid (17-β) dehydrogenase 1 in the second trimester were independent risk factors for PE;[17] and others also showed that HBP, high PI, decreased pregnancy-associated plasma protein-A and low PlGF levels in the first trimester were independent risk factors for not only early PE requiring delivery at <34 weeks but also late PE with delivery at ⩾34 weeks.[18]

The original concept of the two-stage disorder of PE consists of the first stage of reduced placental perfusion followed by the second stage of maternal syndrome.[19,20] It is plausible that the first stage is generated by the impairment of physiologic migration of trophoblasts into spiral arteries.[14] However, it has been criticized because reduced perfusion, posited as secondary to failed remodeling of the maternal vessels supplying the intervillous space, is not sufficient to cause PE.[20,21] In this research, we raised the hypothesis that HBP and BN in the second trimester might additively affect the frequencies of abnormal serum levels of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng in the second and early third trimesters.

Our aim was to evaluate the additive effects of HBP and BN in the second trimester on serum levels of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng in the second and early third trimesters in a prospective pregnant cohort, while stratifying the pregnant cohort into four groups using HBP and BN.

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METHODS

Subjects and procedures

In April 2004, we started a prospective cohort study to make a prediction model of PE using maternal characteristics, BP levels, UAD waveforms and blood markers such as sFlt-1 and PlGF, after approval from the Ethics Committee of our institute. In the prospective cohort study, 1724 pregnant women gave written informed consent until October 2008 (Figure 1). Inclusion criteria were women with singleton pregnancy who sought routine antepartum maternal checkup at our tertiary perinatal center before 24 weeks of gestation. Exclusion criteria were women with multiple pregnancies, and those who sought an initial routine antepartum maternal checkup after 24 weeks of gestation. Blood pressure levels were measured at every maternal checkup. Blood pressure was measured with an Omron HEM-906 automated digital oscillometric sphygmanometer (OMRON Healthcare Japan, Kyoto, Japan), according to standard procedures. UAD was measured at 20–23 weeks of gestation, and BN and the mean PI (mPI) were surveyed. Blood samples were collected twice at 17–25 (mainly 20–23) and 26–32 (mainly 28–29) weeks of gestation. A total of 485 women were excluded from analysis because the UAD was measured only at <16 or $\geqslant 24$ weeks of gestation ($n=42$), BP at 16–23 was not measured ($n=11$), approval to sample blood could not obtained ($n=223$), they delivered at another hospital because of 'Satogaeri bunben' (a traditional ritual (support system) for perinatal women in Japan; 'Satogaeri' means returning to the original family town or house and 'bunben' means delivery), the details of the clinical outcome were not available ($n=82$) and BN at 20–23 weeks was not evaluated ($n=127$). We defined HBP+ as a MBP at 16–23 weeks of $\geqslant 90$ mm Hg, and defined BN+ when notchings at 20–23 weeks were seen in both uterine artery flow velocity waveforms. In the remaining 1239 women, we selected women without either HBP or BN (HBP−BN−, $n=338$) in 800 consecutive pregnant subjects recruited early in this study, whereas we used all women with HBP but not BN (HBP+BN−, $n=272$), BN but not HBP (HBP−BN+, $n=130$) and both HBP and BN (HBP+BN+, $n=60$). These data were used in previous reports.[22]

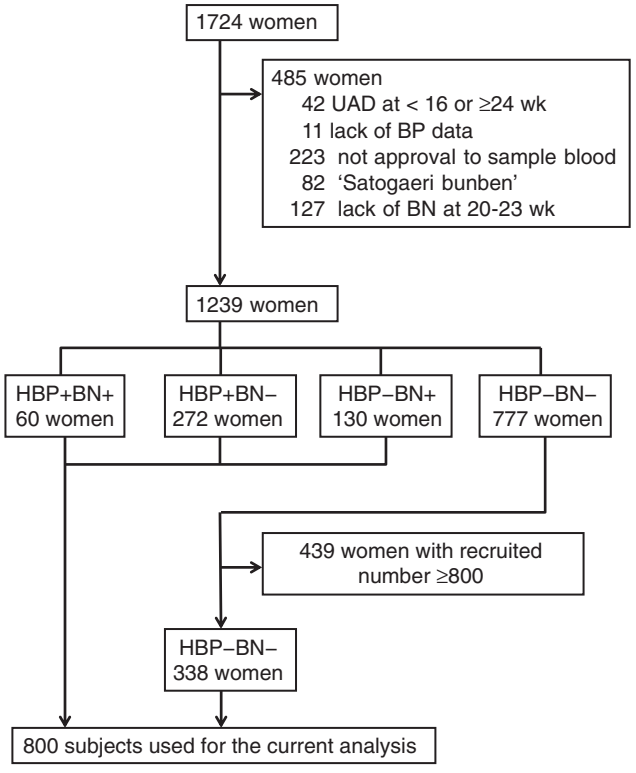

Figure 1 Patient exclusion and selection flowchart for the current analysis. BN, bilateral notching; BP, blood pressure; HBP, high blood pressure; wk, weeks of gestation; UAD, uterine artery Doppler.

Serum samples were centrifuged at 4 °C at 2500 r.p.m. for 15 min, and were stored at −20 °C until use. Enzyme-linked immunosorbent assays for human sFlt-1 (DVR100B; R&D Systems, Minneapolis, MN, USA), PlGF (DPG00; R&D Systems) and sEng (DNDG00; R&D Systems) were performed with a single measurement according to the manufacturer's instructions. The minimal detectable doses in the assays for sFlt-1, PlGF and sEng were 3.5, 7 and 7 pg ml^{-1}, respectively. The intraassay and interassay coefficients of variance for sFlt-1 were 2.6–3.8% and 5.5–9.8%; those for PlGF were 3.6–7.0% and 10.9–11.8%; and those for sEng were 2.8–3.2% and 6.3–6.7%, respectively.

Definition of cutoff values of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng

The details of methods are included in the Supplementary Information. In brief, the cutoff value of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 17–25 weeks was set at 3577, 157, 12.3 and 7.8 ng ml^{-1}, respectively; and cutoff value of each marker at 26–32 weeks was set at 3350, 233, 8.4 and 13.1 ng ml^{-1}, respectively.

Definitions of gestational hypertension (GH) and PE

We defined GH and PE according to the definition and classification of pregnancy-induced hypertension (PIH) (2004) of the Japan Society for the Study of Hypertension in Pregnancy (JSSHP).[23,24] In brief, PE was defined as hypertension with proteinuria occurring after week 20 of gestation but resolving by week 12 postpartum, whereas GH was defined as hypertension without proteinuria occurring after week 20 of gestation but resolving by week 12 postpartum. Hypertension was defined as systolic blood pressure $\geqslant 140$ mm Hg and/or diastolic blood pressure $\geqslant 90$ mm Hg on two occasions at least 4 h apart. Proteinuria was defined as $\geqslant 300$ mg day^{-1} from 24 h urine collection. If only test tape was available, semiquantitative test results of 1+ on 2 occasions at least 1 day apart were considered to constitute a positive result. Superimposed PE was defined as chronic hypertension diagnosed before pregnancy or before week 20 of gestation, with proteinuria emerging afterward. Superimposed PE was included in the category of PE in this study. All diagnoses of PE were decided by KT, who did not know the enzyme-linked ismmunosorbent assay results, and reviewed all the subjects' charts.

Statistics

The results are presented as the mean ± s.d. In the current study, only data before the onset of PE were evaluated. Analysis of variance followed by Dunnett's method was used to compare continuous data in the four groups of HBP−BN−, HBP+BN−, HBP−BN+ and HBP+BN+, whereas HBP−BN− was set as the control. Statistical testing was conducted after logarithmic transformation for serum levels of sFlt-1, PlGF, the sFlt-1/PlGF ratio and sEng. Fisher's exact test followed by the Bonferroni method was used to compare categorical data. All analyses were performed using an IBM SPSS software package (version 21.0, IBM Japan, Tokyo, Japan). $P<0.05$ was considered significant.

RESULTS

The frequency of a past history of GH/PE in women with HBP+BN−, HBP−BN+ and HBP+BN+ was significantly larger than in those with HBP−BN− (controls) (Table 1). The frequency of obesity in women with HBP+BN− and HBP+BN+ was significantly larger than in controls. All PE, PE with onset at <36 weeks, PE with onset at <32 weeks (early-onset PE) and preterm delivery occurred more frequently in women with HBP+BN+ than in controls. Thus, women with HBP+BN+ were the highest-risk group for the occurrence of pregnancy-induced hypertension. In 530 women with recruitment numbers of <801, MBP at 16–23 weeks was not significantly correlated with mPI at 20–23 weeks ($r=0.004$, $P=0.936$); the frequency of HBP in those with BN− was almost the same as that in those with BN+ (25.9% *vs.* 26.7%, $P=0.902$, $n=561$).

Additive effect of HBP and BN on the abnormalities of angiogenesis-related factors

As for the frequencies of abnormal sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 17–25 weeks, the frequency of abnormal sFlt-1 and abnormal sFlt-1/PlGF ratio was not significantly different among the four groups; the frequency of abnormal PlGF in women with HBP − BN −, HBP + BN −, HBP − BN + and HBP + BN + was 3.8%, 6.7%, 8.7% and 18.6%, respectively, and the frequency of abnormal PlGF in those with HBP + BN + was significantly increased than in those with HBP − BN − (controls); and the frequency of abnormal sEng in the four groups was 5.0%, 7.1%, 10.2% and 22.0%, respectively, and the frequency of abnormal sEng in those with HBP + BN + was significantly higher than in controls (Figure 2a).

As for the frequencies of abnormal sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 26–32 weeks, the frequency of abnormal sFlt-1 in women with HBP − BN −, HBP + BN −, HBP − BN + and HBP + BN + was

Table 1 Maternal and infantile characteristics according to the four classes defined by BN at 20–23 weeks and MBP at 16–23 weeks

Characteristics	*Missing values*	*HBP − BN −* (n = *338*)	*HBP + BN −* (n = *272*)	*HBP − BN +* (n = *130*)	*HBP + BN +* (n = *60*)	P-*value*
Women						
Age (years)	0	33.0 ± 5.0	33.7 ± 4.9	**30.6 ± 4.5**	31.5 ± 4.6	<0.001
Nullipara (%)	0	153 (45)	136 (50)	73 (56)	33 (55)	0.140
Race: Japanese (%)	0	336 (99.4)	270 (99.3)	128 (98.5)	59 (98.3)	0.690
Family history of HT (%)	9	101 (30)	87 (32)	28 (22)	22 (37)	0.102
Past history of GH/PE (%)	1	7 (2.1)	**17 (6.3)**	**12 (9.3)**	**7 (12)**	0.001
Current smoking (%)	40	12 (3.8)	15 (6.0)	3 (2.3)	4 (6.7)	0.294
CH (%)	0	0 (0.0)	**19 (7.0)**	0 (0.0)	**9 (15)**	<0.001
Obesity (%)	2	27 (8.0)	**125 (46)**	11 (8.5)	**27 (45)**	<0.001
Prepregnancy BMI (kg m^{-2})	2	21.1 ± 3.0	**25.5 ± 5.3**	21.2 ± 2.7	**25.7 ± 5.4**	<0.001
MBP at 16–23 wk (mm Hg)	0	79 ± 7	**98 ± 7**	80 ± 6	**100 ± 9**	<0.001
mPI at 20–23 wk	35	0.97 ± 0.26	0.94 ± 0.24	**1.35 ± 0.38**	**1.43 ± 0.39**	<0.001
PE (%)	0	3 (0.9)	**15 (5.5)**	5 (3.8)	**11 (18)**	<0.001
PE with onset at <36 wk (%)	0	1 (0.3)	7 (2.6)	2 (1.5)	**7 (12)**	<0.001
Early-onset PE[a] (%)	0	0 (0.0)	2 (0.7)	1 (0.8)	**5 (8.3)**	<0.001
GH (%)	0	4 (1.2)	7 (2.6)	3 (2.3)	**5 (8.3)**	0.010
Infants						
Gestational age at delivery (wk)	0	38.8 ± 1.9	38.6 ± 2.2	38.7 ± 3.4	**36.9 ± 3.7**	<0.001
Preterm delivery (%)	0	26 (7.7)	34 (12.5)	14 (10.8)	**21 (35.0)**	<0.001
Infant birth weight (g)	0	2948 ± 441	2986 ± 562	2810 ± 477	**2429 ± 760**	<0.001

Abbreviations: BMI, body mass index; BN, bilateral notching; CH, chronic hypertension; GH, gestational hypertension; HBP, high blood pressure; HT, hypertension; MBP, mean blood pressure; mPI, mean pulsatility index; PE, preeclampsia; wk, weeks of gestation.
Data are shown as mean ± s.d.
Bold fonts indicate group with significant differences of frequency or mean of the characteristics compared with HBP − BN −.
[a]Early-onset PE was defined as PE with both onsets of hypertension and proteinuria at <32 weeks of gestation.

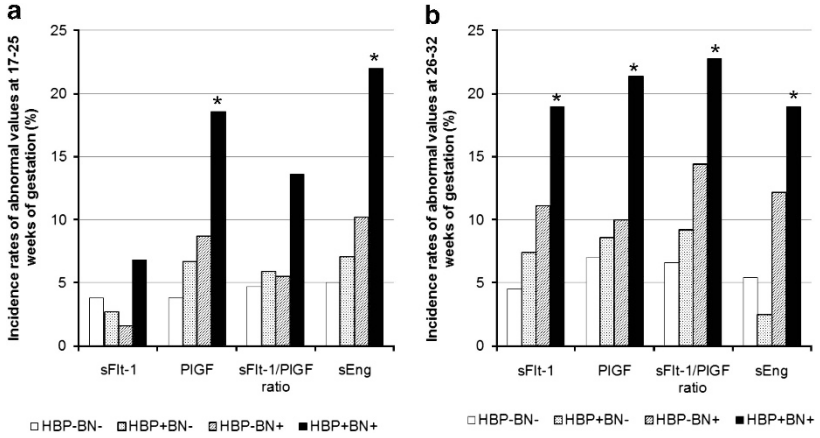

Figure 2 Frequencies of abnormal angiogenesis-related factors at 17–25 and 26–32 weeks of gestation in four classes defined by HBP and BN. Frequencies of abnormal sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 17–25 weeks of gestation (**a**) and 26–32 weeks of gestation (**b**) in women with HBP − BN − (clear bar), HBP + BN − (dotted bar), HBP − BN + (shaded bar) and HBP + BN + (black bar) in the second trimester are shown. The asterisk indicates that the frequency of abnormal values in the category is significantly higher than in the controls (women with HBP − BN −). The cutoff value of abnormal sFlt-1, sFlt-1/PlGF ratio and sEng was the 95th percentile of the respective normal reference range, and the cutoff value of abnormal PlGF was the 5th percentile of the normal reference range. BN, bilateral notching; HBP, high blood pressure; PlGF, placental growth factor; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase 1; wk, weeks of gestation.

4.5%, 7.4%, 11.1% and 19.0%, respectively, and the frequency in those with HBP + BN + was significantly increased than in controls; the frequency of abnormal PlGF in the four groups was 7.0%, 8.6%, 10.0% and 21.4%, respectively, and the frequency in those with HBP + BN + was significantly increased than in controls; the frequency of abnormal sFlt-1/PlGF ratio in the four groups was 6.6%, 9.2%, 14.4% and 22.8%, respectively, and the frequency in those with HBP + BN + was significantly increased than in controls; and the frequency of abnormal sEng in the four groups was 5.4%, 2.5%, 12.2% and 19.0%, respectively, and the frequency in those with HBP + BN + was significantly increased than in controls (Figure 2b).

The correlationship among MBP, mPI and angiogenesis-related factors

We evaluated the correlation coefficients among MBP at 16–23 weeks, mPI at 20–23 weeks and serum levels of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 17–25 and 26–32 weeks (Table 2). There was no correlation between MBP and mPI. MBP was significantly correlated with sFlt-1, PlGF and sFlt-1/PlGF ratio at 17–25 weeks, and with sFlt-1, PlGF and sFlt1/PlGF ratio at 26–32 weeks; and mPI was significantly correlated with sFlt-1, PlGF and sEng at 17–25 weeks, and with sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 26–32 weeks.

Table 2 The correlation coefficients among MBP at 16–23 wks, mPI at 20–23 wks and serum levels of log_{10}sFlt-1, log_{10}PlGF, log_{10}(sFlt-1/PlGF) and log_{10}sEng at 17–25 and 26–32 wks

	MBP	mPI	log_{10}sFlt-1 at 17–25 wks	log_{10}PlGF at 17–25 wks	log_{10} (sFlt-1/PlGF) at 17–25 wks	log_{10}sEng at 17–25 wks	log_{10}sFlt-1 at 26–32 wks	log_{10}PlGF at 26–32 wks	log_{10} (sFlt-1/PlGF) at 26–32 wks	log_{10}sEng at 26–32 wks
MBP	1	−0.018	0.076*	−0.160***	0.180***	0.045	0.129**	−0.150***	0.189***	0.009
mPI	−0.018	1	−0.089*	−0.114**	0.019	0.131***	0.108*	−0.140**	0.168***	0.097*
log_{10}sFlt-1 at 17–25 wks	0.076*	−0.089*	1	0.150***	0.658***	0.286***	0.608***	0.080	0.339***	0.131**
log_{10}PlGF at 17–25 wks	−0.160***	−0.114**	0.150***	1	−0.646***	0.015	−0.003	0.679***	−0.473***	−0.056
log_{10}(sFlt-1/PlGF) at 17–25 wks	0.180***	0.019	0.658***	−0.646***	1	0.210***	0.473***	−0.441***	0.612***	0.143**
log_{10}sEng at 17–25 wks	0.046	0.131***	0.286***	0.015	0.210***	1	0.265***	0.001	0.172***	0.645***
log_{10}sFlt-1 at 26–32 wks	0.129**	0.108*	0.608***	−0.003	0.473***	0.265***	1	−0.097*	0.720***	0.456***
log_{10}PlGF at 26–32 wks	−0.150***	−0.140**	0.080	0.679***	−0.441***	0.001	−0.097*	1	−0.760***	−0.078
log_{10}(sFlt-1/PlGF) at 26–32 wks	0.189***	0.168***	0.339***	−0.473***	0.612***	0.172***	0.720***	−0.760***	1	0.352***
log_{10}sEng at 26–32 wks	0.009	0.097*	0.131**	−0.056	0.143**	0.645***	0.456***	−0.078	0.352***	1

Abbreviations: MBP, mean blood pressure; mPI, mean pulsatility index; PlGF, placental growth factor; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase 1; wks, weeks of gestation.
*$P<0.05$; **$P<0.01$; and ***$P<0.001$.

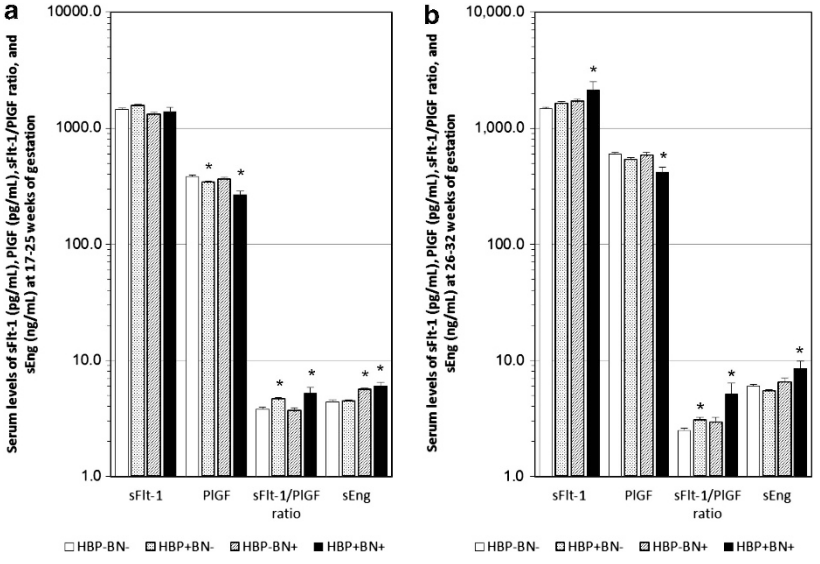

Figure 3 Serum levels of angiogenesis-related factors at 17–25 and 26–32 weeks of gestation in four classes defined by HBP and BN. Serum levels of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 17–25 weeks of gestation (**a**) and 26–32 weeks of gestation (**b**) in women with HBP − BN − (clear bar), HBP + BN − (dotted bar), HBP − BN + (shaded bar) and HBP + BN + (black bar) in the second trimester are shown. The asterisk indicates that the serum level of angiogenesis-related factors in the category is significantly higher than in the controls (women with HBP − BN −). BN, bilateral notching; HBP, high blood pressure; PlGF, placental growth factor; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase 1; wk, weeks of gestation.

The comparisons of raw values of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng

As for the serum levels of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 17–25 weeks, sFlt-1 was not significantly different among women with HBP −BN− (controls), HBP + BN −, HBP −BN + and HBP + BN +; the levels of PlGF in those with HBP + BN − and those with HBP + BN + were significantly larger than in the controls; the levels of sFlt-1/PlGF ratio in those with HBP + BN − and those with HBP + BN + were significantly larger than in the controls; and the levels of sEng in those with HBP −BN + and those with HBP + BN + were significantly larger than in the controls (Figure 3a).

As for the serum levels of sFlt-1, PlGF, sFlt-1/PlGF ratio and sEng at 26–32 weeks, the level of sFlt-1 in those with HBP + BN + was significantly larger than in the controls; the level of PlGF in those with HBP + BN + was significantly larger than in the controls; the levels of sFlt-1/PlGF ratio in those with HBP + BN − and those with HBP + BN + were significantly larger than in the control; and the level of sEng in those with HBP + BN + was significantly larger than in the controls (Figure 3a).

DISCUSSION

In this study, we found the following novel findings. First, high MBP levels and BN were additively implicated in the appearances of abnormal PlGF and abnormal sEng at 17–25 weeks. Second, high MBP levels and BN were additively implicated in the appearances of not only abnormal PlGF and abnormal sEng but also abnormal sFlt-1 and abnormal sFlt-1/PlGF ratio at 26–32 weeks. These two findings suggested that a predisposition to hypertension and hypoperfusion to the placenta in the second trimester may additively interact to increase the release of antiangiogenic factors from the placenta into the maternal circulation, and may finally increase the probability of the later occurrence of PE.

Redman[19] originally introduced the concept that PE may be a two-stage disorder: the first stage is reduced placental perfusion that leads to the second stage, maternal syndrome. It has been believed that the first stage is generated by the impairment of physiologic migration of trophoblasts into spiral arteries, a phenomenon that is reflected by abnormal UAD in the first and second trimesters, such as BN, high resistance index, high PI or high notch depth index.[4–6,17,18] However, it has been criticized because reduced perfusion, posited as secondary to failed remodeling of the maternal vessels supplying the intervillous space, is not sufficient to cause PE.[20,21] Maternal constitutional factors such as genetic susceptibility, obesity, diabetes and diet might be necessary to interact with reduced placental perfusion to lead to PE;[20,21] or oxidative stress, endoplasmic reticulum stress and inflammatory stress might be necessary to interact with reduced placental perfusion to lead to overt PE.[25] To the best of our knowledge, no researchers have proposed that not only impairment of the physiological migration of trophoblasts into the spiral arteries, but also increased blood pressure might affect the maternal vessels supplying the intervillous space, although Roberts and Hubel[20] have proposed that maternal constitutional factors, such as genetic, obesity and diabetes, could also stimulate abnormal placentation. In our current research, we ascertained that HBP and BN in the second trimester additively affected the abnormal increases of sFlt-1 and sEng in the maternal circulation before the onset of PE, suggesting that a predisposition to hypertension and hypoperfusion to the placenta in the second trimester may synergistically interact to increase the release of antiangiogenic factors from the placenta into the maternal circulation, and may finally increase the probability of the later occurrence of PE.

In conclusion, we found that in pregnant women with both HBP and BN in the second trimester, serum levels of sFlt-1/PlGF and sEng in the second and early third trimesters were significantly increased. However, we do not know why relatively high blood pressure induced the release of angiogenesis-related factors from the placenta into the maternal circulation. If maternal blood pressure is controlled more strictly, are the effects of HBP on the increase of angiogenesis-related factors lessened? The comparison of women with chronic hypertension with and without strict blood level controls might answer our question.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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