ORIGINAL ARTICLE

Increased oxygen free radical production during pregnancy may impair vascular reactivity in preeclamptic women

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To determine whether enhanced oxidative stress during pregnancy impairs vascular endothelial function and improves after delivery in preeclamptic women, we measured serum parameters of oxidative stress and endothelial function during pregnancy and 1 month after delivery in women with or without preeclampsia. We evaluated 18 participants with uncomplicated pregnancies, 11 with mild preeclampsia and 13 with severe preeclampsia. The plasma concentrations of reactive oxygen metabolite derivatives (d-ROMs) were measured, and the biological antioxidant potential (BAP) was determined to evaluate the oxygen free radicals and antioxidants, respectively. Flow-mediated vasodilation (FMD) was also assessed as a marker of endothelial function. FMD was decreased significantly in both preeclamptic groups compared with control during pregnancy. FMD did not change after delivery in the control group, but it significantly increased after delivery in both the mildly and severely preeclamptic groups, nearing control levels 1 month after delivery (mild, 6.5 ± 3.6 – $9.0 \pm 3.5\%$; severe, 4.3 ± 3.3 – $9.7 \pm 2.6\%$). No changes in d-ROM concentrations were observed in the control group; however, the concentrations in both the mildly and severely preeclamptic groups significantly decreased to normal levels 1 month after delivery (mild, 562.0 ± 106.5 – 430.5 ± 90.5 CARR U (Carratelli units); severe, 681.0 ± 239.0 – 411.8 ± 69.7 CARR U). The plasma BAP levels did not change significantly in all three groups. A negative correlation between FMD and d-ROM concentrations was observed in the preeclamptic group, but not in the control group ($r = -0.497$; $P < 0.05$). Our findings indicated that enhanced oxidative stress during pregnancy may impair endothelial function and improve after delivery in preeclamptic women.

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INTRODUCTION

Preeclampsia, a complex generalized syndrome in pregnancy characterized by hypertension and proteinuria, remains a major cause of maternal and fetal morbidity and mortality.¹ Endothelial dysfunction is considered a basic pathological event that occurs with preeclampsia.² This endothelial damage is mediated in part by oxidative stress that results from increased oxygen free radicals and/or decreased antioxidant levels.³ Endothelial injury reduces vasodilator (that is, prostacyclin and nitric oxide (NO)) production and increases vasoconstrictor (that is, endothelin-1 and thromboxane) production, thereby resulting in vasospasms.

The high maternal and fetal oxygen demand during pregnancy increases oxidative metabolism, which promotes free radical generation.⁴ Superoxide anions produced by the placental mitochondria appear to be an important source of oxidative stress, which contributes to an overall increase in maternal blood and placental lipid peroxidation in preeclamptic women.^{5–8} We previously suggested that increased oxygen free radicals may originate from the

placenta in preeclamptic women as these free radicals decrease after delivery.⁹

The measurement of flow-mediated vasodilation (FMD) of conduit arteries is a widely used test of endothelial function.^{10,11} Endothelial dysfunction is an early event in atherogenesis and exists in patients with coronary artery disease and other cardiovascular risk factors.^{10,12} An increase in vascular shear stress, which results from an increase in blood flow and the concomitant release of NO, has been identified as a mechanism for FMD.^{13,14} We previously found that FMD of the brachial artery, a known marker of vascular endothelial function, is decreased in preeclamptic women.^{15,16}

Pre-existing chronic hypertension, obesity, diabetes mellitus and systemic lupus erythematosus may initiate vasospasms secondary to endothelial dysfunction, and endothelial dysfunction does not completely improve after delivery. Pregnancies in patients with these pre-existing conditions have a greater risk of preeclampsia, which frequently becomes apparent during pregnancy. However, it is unclear whether enhanced oxidative stress during pregnancy impairs vascular

endothelial function and whether it improves after delivery in preeclamptic women without these pre-existing conditions. To address this, we measured serum parameters of oxidative stress and endothelial function during pregnancy and 1 month after delivery in women with or without preeclampsia.

METHODS

Study participants

Between 1 April 2008 and 31 March 2010, we recruited 11 participants with mild preeclampsia, 13 participants with severe preeclampsia and 18 normal participants with uncomplicated pregnancies. The selection criteria for participants with mild preeclampsia included a systolic blood pressure (BP) ≥ 140 and < 160 mmHg or a diastolic BP ≥ 90 and < 110 mmHg after 20 weeks of gestation and proteinuria (≥ 300 mg and < 2 g protein per 24 h). The selection criteria for participants with severe preeclampsia included a systolic BP ≥ 160 mmHg or diastolic BP ≥ 110 mmHg after 20 weeks of gestation and proteinuria (≥ 2 g protein per 24 h). None of the preeclamptic participants showed signs of hemolysis, elevated liver enzymes, and low platelet syndrome. Participants with uncomplicated pregnancies, matched for maternal and gestational age, were used as controls. Each control group participant had a normal pregnancy course and full-term delivery. None of the participants smoked, used caffeine or alcohol, or had a history of thyroid disease, liver disease, diabetes mellitus, hypertension, hyperlipidemia, or were taking any medication known to influence lipoprotein metabolism. In the present study, all participants delivered by cesarean section before the onset of labor. All control group participants underwent cesarean section due to a previous cesarean section or breech presentation. Written informed consent was obtained from each participant. This study was approved by the Ethics Committee of Aichi Medical University School of Medicine.

Derivatives of reactive oxygen metabolites and biological antioxidant potential (BAP)

Blood samples were collected from each participant between 0900 hours and 1100 hours after a 12-h fasting period before delivery in the third quarter and 1 month after delivery. Serum derivatives of reactive oxygen metabolites (d-ROMs), a marker of oxidant status, and the serum BAP were analyzed using a Free Radical Analytical System 4 (Diacron, Grosseto, Italy).^{17–20} Organic hydroperoxide serum measurements were taken to determine the levels of free radicals and the source. Under acidic conditions, hydroperoxides react with iron, a transition metal that is liberated from proteins, and they are converted into alkoxyl and peroxy radicals. These radicals can oxidize an additive to its corresponding radical cation species. The concentrations of these persistent species were measured by spectrophotometry (505 nm). The results for all d-ROM measurements were expressed in Carratelli units (CARR U).

The BAP serum levels were also measured to determine the antioxidant levels. The BAP test is based on the ability of a colored solution containing a source of ferric ions bound to a special chromogenic substrate to decolorize when ferric ions are reduced to ferrous ions, which occurs when a reducing/antioxidant source is added. The concentration is then measured by spectrophotometry (505 nm). In the present study, an imbalance between increased oxygen free radicals and the antioxidative processes was determined and used to calculate d-ROMs and BAP concentrations in both maternal and postpartum blood.

Endothelial function

All participants were required to rest in a supine position for 10 min before the study. A high-resolution, Doppler ultrasonography machine (VolusonE8, GE Healthcare, Nagoya City, Japan) with a 10-MHz transducer was used to image the right brachial artery and measure the vasodilatory responses. A nontortuous segment of the brachial artery was scanned longitudinally 4–5 cm above the elbow, as this was the region where the clearest image could be obtained. Once the adequate transducer position was determined, the skin was marked and the arm was kept in a constant position for the remainder of the study. After baseline images of the brachial artery were obtained and the arterial flow velocity was determined, a BP cuff encircling the proximal portion of the arm

was inflated to 250 mmHg for 5 min and then suddenly deflated. Increased blood flow following sudden cuff deflation (that is, reactive hyperemia) results in flow-mediated vasodilatation (FMD).²¹ The arterial flow velocity was determined again and, 1 min after cuff deflation, the brachial artery was imaged. BP and heart rate were monitored and recorded throughout the study. The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia ('m line') at end diastole, incident with the R wave on a continuously recorded electrocardiogram. The diameters for four cardiac cycles were determined from the images and the measurements were averaged. All scans were recorded for further analysis. The vessel diameters were measured without prior knowledge of the participant information. FMD was calculated as the percent increase in arterial diameter during hyperemia and used as an index of endothelium-dependent vasodilation.²² Intraobserver and interobserver variability for repeat measurements were 0.03 ± 0.02 and 0.05 ± 0.03 mm, respectively, and the variability for FMDs measured on two separate days was $2.1 \pm 0.9\%$.

Statistical analyses

SPSS Statistical Software, version 14.0 for Windows (SPSS Inc., Chicago, IL, USA), was used for all statistical analyses. The data are expressed as the mean $\pm$ s.d. The patient characteristics, serum d-ROM and BAP concentrations and FMD were compared using a one-way analysis of variance, followed by Scheffé's test. The patient characteristics, serum d-ROM and BAP concentrations and FMD before and post delivery were compared using the Student's paired *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Maternal and gestational age at the time of the study were similar between both preeclamptic groups (that is, mildly and severely) and the control group. The body mass index was significantly higher in the mildly preeclamptic group compared with the severely preeclamptic and control groups. In addition, plasma uric acid levels were significantly elevated during pregnancy in both preeclamptic groups, but they were higher in the severely preeclamptic group. Hematocrit, aspartate aminotransferase and plasma creatine levels during pregnancy were significantly elevated in the severely preeclamptic group but not in the mild preeclamptic group. In addition, the platelet counts during pregnancy were significantly elevated in the mildly preeclamptic group but not in the severely preeclamptic group. Hematocrit and platelet counts, liver enzyme, blood urea nitrogen and creatine plasma levels measured 1 month after delivery were similar for all three groups (Table 1).

No significant changes in d-ROM plasma concentrations before and 1 month after delivery were observed in the control group. However, those concentrations were significantly lower in both preeclamptic groups, reaching control levels 1 month after delivery (mild, 562.0 ± 106.5 – 430.5 ± 90.5 CARR U; severe, 681.0 ± 239.0 – 411.8 ± 69.7 CARR U; Figure 1). The BAP concentrations before and 1 month after delivery were similar in all three groups (Figure 1).

The systolic BP measured 1 month after delivery was significantly higher in both preeclamptic groups. In contrast, the diastolic BP improved to normal levels in both preeclamptic groups. Finally, no significant difference was found in the brachial artery diameter and blood flow velocity during pregnancy and 1 month after delivery in all three groups. The percent increase in blood flow velocity promoted by reactive hyperemia did not change significantly (Table 2). FMD decreased significantly in both preeclamptic groups compared with the control during pregnancy. FMD in the control group remained the same after delivery, but it increased significantly to control levels 1 month after delivery in both preeclamptic groups (mild, 6.5 ± 3.6 – $9.0 \pm 3.5\%$; severe, 4.3 ± 3.3 – $9.7 \pm 2.6\%$; Figure 2). A negative correlation between FMD and d-ROM concentrations was observed in the

Table 1 Subjects characteristics

	Normal (n = 18)		Mild preeclampsia (n = 11)		Severe preeclampsia (n = 13)	
	During pregnancy	1 month after delivery	During pregnancy	1 month after delivery	During pregnancy	1 month after delivery
Age (years)	33.5 ± 4.6	—	31.3 ± 5.4	—	31.5 ± 5.2	—
Gestational age at test (weeks)	34.6 ± 5.0	—	34.7 ± 3.3	—	33.3 ± 2.9	—
BMI (kg m ⁻²)	19.7 ± 2.2	—	23.5 ± 7.6 ^a	—	22.2 ± 3.9	—
Parity		1.9 ± 1.1		1.6 ± 0.8		1.6 ± 1.4
Hematocrit (%)	31.7 ± 1.8	36.6 ± 3.4	32.3 ± 2.9	36.3 ± 3.5 ^g	38.4 ± 7.7 ^{b,c}	35.3 ± 3.3
Platelet (× 10 ⁴)	24.3 ± 5.5	28.1 ± 10.8	31.1 ± 9.2 ^{a,e}	31.4 ± 12.8	24.9 ± 8.2	37.9 ± 17.5 ^g
AST (IU l ⁻¹)	16.7 ± 4.0	21.4 ± 6.9	13.8 ± 3.7	18.5 ± 6.2	20.0 ± 4.4 ^{a,d}	20.7 ± 6.6
ALT (IU l ⁻¹)	10.6 ± 5.0	16.2 ± 2.2 ^f	8.2 ± 3.2	21.0 ± 11.8 ^g	12.7 ± 9.0	21.2 ± 10.3 ^g
BUN (mg dl ⁻¹)	7.8 ± 2.4	11.5 ± 3.3	7.4 ± 2.1	10.9 ± 3.3 ^g	12.5 ± 2.9 ^{b,d}	10.3 ± 2.4 ^f
Creatinine (mg dl ⁻¹)	0.44 ± 0.06	0.53 ± 0.07	0.50 ± 0.10	0.57 ± 0.08 ^f	0.62 ± 0.11 ^{b,d}	0.58 ± 0.08 ^f
Uric acid (mg dl ⁻¹)	4.0 ± 0.8	4.4 ± 0.6	4.9 ± 1.1 ^a	5.6 ± 1.2 ^{a,f}	6.5 ± 1.4 ^{b,d}	5.3 ± 0.9 ^g

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen.

Data are expressed as mean ± s.d.

^aP < 0.05, ^bP < 0.01 as compared with normal.

^cP < 0.05, ^dP < 0.01 as compared with mild preeclampsia.

^eP < 0.05 as compared with severe preeclampsia.

^fP < 0.05, ^gP < 0.01 as compared with during pregnancy.

Figure 1 Concentrations of derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) in maternal blood during pregnancy and 1 month after delivery in the control group and both preeclamptic women. Open, closed and diagonally hatched columns represent healthy women with uncomplicated pregnancies, mild preeclamptic women and severe preeclamptic women, respectively. During and after indicate during pregnancy and 1 month after delivery, respectively. N.S. indicates not significant. Data are expressed as mean ± s.d.

Table 2 Blood pressure, heart rate, artery diameter and blood flow

	Normal		Mild preeclampsia		Severe preeclampsia	
	During pregnancy	1 month after delivery	During pregnancy	1 month after delivery	During pregnancy	1 month after delivery
Systolic BP (mm Hg)	111.9 ± 14.0	114.4 ± 8.7	141.9 ± 11.6 ^b	131.7 ± 10.3 ^b	164.8 ± 20.8 ^{b,c}	126.1 ± 9.8 ^{a,d}
Diastolic BP (mm Hg)	75.0 ± 6.9	73.4 ± 8.8	84.9 ± 9.7 ^a	80.2 ± 7.1	103.8 ± 8.7 ^{b,c}	80.2 ± 8.1 ^e
Heart rate (beats per minute)	72.7 ± 3.9	72.6 ± 6.3	73.0 ± 2.8	73.2 ± 10.7	78.1 ± 7.5	77.9 ± 6.6
Baseline diameter (mm)	3.4 ± 0.3	3.1 ± 0.3	3.6 ± 0.3	3.1 ± 0.2	3.7 ± 0.1	3.2 ± 0.2 ^e
Baseline flow (ml min ⁻¹)	183 ± 89	116 ± 45 ^d	209 ± 79	115 ± 47 ^d	218 ± 87	149 ± 53 ^d
Hyperemic flow (ml min ⁻¹)	346 ± 98	312 ± 149	417 ± 108	317 ± 96	368 ± 117	408 ± 219

Abbreviation: BP, blood pressure.

Data are expressed as mean ± s.d.

^aP < 0.05, ^bP < 0.01 as compared with normal.

^cP < 0.01 as compared with mild preeclampsia.

^dP < 0.05, ^eP < 0.01 as compared with during pregnancy.

Figure 2 Flow-mediated vasodilation during pregnancy and 1 month after delivery in the control group and both preeclamptic women. Open, closed and diagonally hatched columns represent healthy women with uncomplicated pregnancies, mild preeclamptic women, and severe preeclamptic women, respectively. N.S. indicates not significant. Data are expressed as mean $\pm$ s.d.

Figure 3 Relationship between concentrations of derivatives of reactive oxygen metabolites (d-ROMs) and flow-mediated vasodilation during pregnancy in the control group and preeclamptic women. Open circles, closed circles and closed triangles represent healthy women with uncomplicated pregnancies, mild preeclamptic women and severe preeclamptic women, respectively. $r = -0.497$; $P < 0.05$.

preeclamptic group, but not in the control group ($r = -0.497$; $P < 0.05$) (Figure 3).

DISCUSSION

Oxygen free radical production is commonly increased in women with preeclampsia. Although a physiological balance between lipid peroxides and antioxidative processes is maintained during a normal pregnancy,²³ an imbalance may occur, thereby increasing the oxidative stress in preeclamptic women. In the present study, we found that d-ROM concentrations in the blood were significantly higher in the severely preeclamptic group, but not in the mildly preeclamptic group, compared with the control group. In contrast, BAP concentrations did not change significantly across all three groups. These results indicate that the balance between oxygen free radicals

and antioxidative processes is maintained in mildly preeclamptic women, but not in severely preeclamptic women. The results also indicate that this imbalance is dependent on increased oxygen free radical production and not on decreased antioxidative processes. Furthermore, we investigated whether enhanced oxidative stress improved after delivery. We demonstrated that plasma d-ROM levels 1 month after delivery in the severe preeclamptic group normalized to control levels. These results indicate that oxidative stress is greater during pregnancy but that it normalizes after delivery in severely preeclamptic women. Although the d-ROM concentrations in the mildly preeclamptic and control groups were not significantly different during pregnancy, the levels in the mildly preeclamptic group were reduced 1 month after delivery. These results indicate that oxidative stress is slightly higher during pregnancy in mildly preeclamptic women. We believe that the severity of preeclampsia during pregnancy increases with oxidative stress.

NO is an endothelium-derived relaxing factor that is released in response to increased blood flow during reactive hyperemia. Because several NO synthase inhibitors inhibit endothelium-dependent vasodilation, FMD appears to represent a vasodilation-dependent effect mediated by endothelium-derived NO.¹⁴ In the present study, a negative correlation between FMD and d-ROM concentrations was observed in the preeclamptic group. We also found that d-ROMs and FMD in both preeclamptic groups normalized 1 month after delivery, suggesting that oxidative stress during pregnancy may impair endothelial function. In contrast, Hamad *et al.*²⁴ reported that FMD and endothelium-independent glycerin trinitrate-induced dilatation, a marker of vascular muscle cell function, are decreased even 1 year after a preeclamptic pregnancy. These results indicate that endothelial dysfunction remains after delivery, or that after improving post delivery, it decreases again soon thereafter. In their study, endothelial dysfunction that remains after delivery is at an advanced stage due to decreased glycerin trinitrate-induced dilatation, a marker of vascular muscle cell function. Indeed, FMD was lower in their study than in ours. The authors themselves note that the possibility of generalized impairment in vascular muscle cell responsiveness contributing to the observed low FMD cannot be excluded. In our view, the endothelial and vascular muscle cell dysfunction may have preexisted before pregnancy as chronic hypertension in their study. We speculate that if conditions such as obesity, dysregulation of adipocytokines, and oxidative stress persist after delivery, they may impair endothelial function in the future. Indeed, the body mass index was significantly higher in the preeclamptic group compared with the control group 1 year after a preeclamptic pregnancy in their study.²⁴ Consistent with this, in our preliminary study, endothelial dysfunction failed to improve, and it remained after delivery in pregnant women with pre-existing conditions such as chronic hypertension.

Yinon *et al.*²⁵ reported that FMD was significantly reduced in women with a history of early-onset preeclampsia and normotensive intrauterine fetal growth restriction, but not in women with a history of late-onset preeclampsia. In a previous study, we defined preeclampsia with an onset before the 32nd week of gestation as early-onset preeclampsia, and preeclampsia with an onset after the 32nd week was defined as late-onset preeclampsia. We found that FMD in patients with early-onset preeclampsia was significantly lower than in those with late-onset preeclampsia.¹⁶ This indicates that endothelial dysfunction is more severe in early-onset preeclampsia than in late-onset preeclampsia. Given that FMD was not tracked long term after delivery in the present study, further studies will be needed to address this possibility.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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