Flow-Mediated Vasodilation and Plasma Fibronectin Levels in Preeclampsia

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Abstract—To clarify the vascular endothelial function in pregnant women with hypertensive disorders, we assessed the flow-mediated vasodilation in the radial artery and compared it with plasma fibronectin levels. We determined flow-mediated vasodilation by measuring the change in radial artery diameter during hyperemia in 58 normal pregnant women, 22 preeclamptic pregnant women, and 15 pregnant women with chronic hypertension. In 41 of the 95 pregnant women, we measured the plasma fibronectin levels. Flow-mediated vasodilation in preeclamptic women was significantly less than that in normal pregnant women (P<0.001). In chronic hypertensive women, flow-mediated vasodilation was significantly less than that in normal pregnant women (P<0.001) but more than that in preeclamptic women (P<0.001). Flow-mediated vasodilation showed significant negative correlation with plasma fibronectin levels (P<0.001, r=0.73). Our results indicate that the endothelial function can be noninvasively assessed in pregnant women with hypertensive disorders by measuring the flow-mediated vasodilation of the radial artery with high-resolution ultrasound. (Hypertension. 2000;36:400-404.)

Key Words: endothelium ■ vasodilation ■ preeclampsia ■ hypertension, pregnancy ■ fibronectins

Recent evidence suggests that vascular endothelial dysfunction might play an important role in the pathogenesis of preeclampsia.1,2 There are several suggested methods for assessment of the endothelial damage, including the evaluation of endothelial cell–derived biochemical markers. Endothelium-related serum factors such as cellular fibronectin and the von Willebrand factor have been shown to be abnormal in preeclampsia.3–5

Another method is the assessment of endothelium-dependent vascular relaxing function. An important functional consequence of endothelial dysfunction is the inability to release endothelium-derived relaxing factor (EDRF).6 EDRF is released in response to various pharmacological and physiological stimuli7,8 and results in increased blood flow. Under physiological conditions, increased blood flow subsequent to the release from temporal occlusion of the peripheral artery causes vasodilation, which is called reactive hyperemia.8 With high-resolution ultrasound, it is possible to objectively observe the vascular changes associated with reactive hyperemia.9–11

To clarify the endothelial function in pregnancy complicated with hypertensive disorders, we noninvasively assessed the flow-mediated vasodilation in the radial artery with a recently developed 30-MHz mechanical linear probe. Previously, we reported flow-mediated vasodilation in smaller number of pregnant subjects.12 In our current report, we measured plasma fibronectin level, one of the endothelial cell–injury markers, and determined the relationship between vasodilation and fibronectin level.

Methods

We examined a total of 95 Japanese women, including 58 normal pregnant women, 22 pregnant women with preeclampsia, and 15 pregnant women with chronic hypertension. The diagnosis of preeclampsia and chronic hypertension was established on the basis of the criteria of the Committee on Terminology of the American College of Obstetricians and Gynecologists.13 All the subjects were nonsmokers and received no antihypertensive agents before the measurement. The majority of chronic hypertensive women (20 of 22) were receiving low-salt dietary therapy. The Table shows the clinical data for these 95 women. The mean gestational age of the preeclampsia group was significantly later than that of the normal pregnancy or chronic hypertension group because preeclampsia subjects entered only after 20 weeks of gestation.

Images of the radial artery in 95 pregnant women were obtained longitudinally with a recently developed 30-MHz mechanical linear probe and the Aloka SSD-550 system. The transducer was positioned perpendicular to the vessel, coupled to the skin with the use of ultrasound gel and very light pressure to avoid vessel compression with the transducer. In each study, we confirmed the clear visualization of the 3 layers of the vessel wall, including the m-lines (the interface between media and adventitia) in both near and far walls. When clear visualization of the layers was confirmed, the probe was fixed with a steel flexible arm. Adequate scans were obtained in all cases.

Each subject lay at rest for at least 5 minutes before the scan. A cuff with 140-mm width was placed on the upper arm and inflated to 30 mm Hg above the systolic blood pressure for 5 minutes. The radial artery diameter was measured before inflation (baseline) and after deflation of the cuff. Inflation of the cuff was started within 1 minute after the measurement of baseline radial artery diameter in each case. Imaging of the artery was performed for 6 minutes after cuff deflation. Radial artery diameter is defined as the distance from the far side of the m-line in the near wall to the near side of the far wall (Figure 1). Measurements were taken before cuff inflation (baseline) and 0.5, 1, 1.5, 2, 3, 4, 5, and 6 minutes after cuff deflation.
at end diastole. Flow-mediated vasodilation was determined by calculating the change in the radial artery diameter (maximum radial artery diameter/baseline radial artery diameter)×100%.

We measured the plasma fibronectin levels in 41 of the 95 subjects with antigen-antibody reaction of the turbidimetric immunoassay method. Blood samples were taken within 24 hours after measurement of the radial artery diameter.

ANOVA was used to compare the mean values. Populations were compared with use of the $\chi^2$ test. Correlation coefficients were compared with use of the $r$ correlation test. The significance of the difference among the correlation coefficients was assessed with $t$ correlation test; $P<0.01$ was considered statistically significant.

**Results**

The baseline radial artery diameter under the conditions of normal pregnancy, preeclampsia, and chronic hypertension was $2.3\pm0.4$, $2.4\pm0.5$, and $2.5\pm0.4$ mm, respectively. No significant differences were seen among these 3 groups.

Figure 2 shows the changes in mean percent dilation of radial artery before and after the cuff deflation. Maximum dilation was obtained 1 minute after cuff deflation. No significant difference was shown between baseline diameter and radial artery diameter after 4, 5, and 6 minutes: that is, radial artery diameter returned to the baseline diameter 4 minutes after the cuff deflation.

Figure 3 shows the relationship between gestational age and radial artery vasodilation. In the normal pregnant subjects, vasodilation was gradually increased according to the progression of gestational age. However, preeclamptic women persistently had little vasodilation. Pregnant women with preeclampsia showed significantly less vasodilation in the radial artery (7.9±3.0%) than did normal pregnant women (17.4±4.2%, $P<0.001$) or chronic hypertensive pregnant women (13.9±2.2%, $P<0.001$). The chronic hypertensive pregnant women showed significantly less vasodilation than did the normal pregnant women ($P<0.001$).

Figure 4 shows the mean fibronectin levels in each group. The preeclamptic subjects showed significantly higher levels of plasma fibronectin than did the normal pregnant or chronic hypertensive pregnant women. The relationship between vasodilation and plasma fibronectin is shown in Figure 5. A significant negative correlation was seen between vasodilation and plasma fibronectin ($P<0.001$); the correlation coef-

![Figure 1. Longitudinal scan made with a 30-MHz transducer of the radial artery of a normal pregnant woman. The double-line patterns, including m-lines (small arrows), are visible. Radial artery diameter is defined as the distance from the far side of the m-line in the near wall to the near side of the far wall (long arrow).](image)

![Figure 2. Changes in percent increase in radial artery diameter between before and after cuff dilation. *$P<0.001$ vs baseline by ANOVA. †Not significant.](image)
ficent (Pearson’s correlation coefficient, \( r \)) was 0.73. In each group, a significant negative correlation was seen between vasodilation and plasma fibronectin (normal \( P < 0.01, r = 0.45 \); chronic hypertension \( P < 0.001, r = 0.74 \); preeclampsia \( P < 0.001, r = 0.77 \)). A significant negative correlation was seen (\( P < 0.001 \)) between vasodilation and mean arterial pressure; the correlation coefficient between vasodilation and mean arterial pressure was 0.56, which was lower than that between vasodilation and plasma fibronectin, but the difference was not significant (\( P = 0.14 \)). In the preeclamptic group, a significant negative correlation was seen between vasodilation and mean arterial pressure (\( P < 0.01, r = 0.52 \)). However, in the normal and chronic hypertensive groups, no significant correlation was seen (normal \( P = 0.1, r = 0.21 \); chronic hypertension \( P = 0.06, r = 0.51 \)).

**Discussion**

Vascular endothelial damage has recently been thought to be a central pathogenic feature of preeclampsia.\(^1\)\(^,\)\(^2\) To assess the endothelial damage, several methods have been used. One of the methods is the assessment of the endothelium-dependent vascular relaxing function. Another method is the assessment of the endothelial injury by measuring biochemical markers such as fibronectin and the von Willebrand factor.

Under physiological conditions, the endothelium of peripheral vessels produces vascular relaxing factors (EDRF). EDRF was first described by Furchgott and Zawadzki.\(^6\) EDRF is produced by the endothelial cells and acts on vascular smooth muscles; it is released in response to various chemical and physical stimuli. An important functional consequence of endothelial dysfunction is the inability to release EDRF. Endothelial dysfunction has been shown in response to various pharmacological and physiological stimuli through the use of intravascular catheterization.\(^7\)\(^,\)\(^8\) These invasive studies are not suitable for the assessment of endothelial function during pregnancy.

One stimulus that releases EDRF is increased blood flow.\(^15\)\(^-\)\(^17\) Celermajer et al\(^9\),\(^10\) reported on noninvasive assessment through the measurement of postocclusion effects on the brachial artery during hyperemia with the use of high-resolution ultrasound. They showed that flow-mediated vasodilation of brachial artery is abnormal in smokers and in persons with hypercholesterolemia. Lieberman et al\(^11\) reported that endothelium-dependent vasodilation is impaired in postmenopausal women and improved with estrogen replacement therapy. Several other investigators reported on the noninvasive assessment of flow-mediated vasodilation.\(^18\)\(^-\)\(^21\) However, all of these studies were conducted with nonpregnant subjects.

In pregnant subjects, previous studies have reported that endothelium relaxing function is reduced in pregnant women with preeclampsia. Ashworth et al\(^22\) reported that markedly reduced endothelium-dependent relaxation in response to bradykinin was found in the myometrial arteries of preeclamptic women. Cockell and Poston\(^23\) reported that the arteries of preeclamptic women showed less flow-mediated vasodilation. However, these studies were in vitro studies with biopsy samples obtained from pregnant women during cesarean section.

In the present study, we indirectly assessed endothelial function in pregnant women who had hypertensive disorders with the use of high-resolution ultrasound. Flow-mediated vasodilation in preeclamptic women was significantly less than that of normal pregnant or chronic hypertensive women, and this finding is consistent with earlier studies. We used a recently developed 30-MHz ultrasound transducer, whereas previous studies with nonpregnant subjects have used 7.0- or 7.5-MHz high-resolution ultrasound transducer. Sorensen et al\(^24\) claimed that a 7.0-MHz transducer provided visualization of the brachial artery and flow-mediated vasodilation.\(^24\) We, however, previously reported the serious limitations of the
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7.5-MHz transducer in detection of the peripheral arteries. Instead of B-mode ultrasound, others have used an A-mode ultrasonic echo-tracking device with a 10-MHz transducer for the assessment of endothelial function in their report. Joannides et al used an A-mode echo-tracking system in the radial artery and reported the relationship between nitric oxide (an EDRF) and flow-mediated vasodilation. We have never used this device, but in general, B-mode ultrasound images provide more directly recognizable visualization. In the current study, we used a recently developed 30-MHz ultrasound transducer, which enabled us to obtain clear visualization of the radial artery in all cases.

A number of researchers have reported increased levels of endothelial cell–derived biochemical markers in preeclampsia women. Fibronectin, an adhesive glycoprotein, is thought to be one of the cell-injury markers. Shaarawy and Didy compared the levels of various biomarkers (thrombomodulin, plasminogen activator inhibitor type 1, and fibronectin) and concluded that fibronectin was the most predictive of the three. Several authors reported increased plasma levels of fibronectin as a predictor of preeclampsia. In our current study, the plasma levels of fibronectin in preeclamptic women were significantly higher than those in women with normal pregnancy or chronic hypertension. This supports the potential predictive value of fibronectin as a marker of preeclampsia.

We compared the 2 methods (endothelium-dependent vascular relaxing function and a marker for preeclampsia) to assess the endothelial damage in preeclamptic women. Plasma total fibronectin is not exactly a marker of endothelial cell injury but is reported to be a predictive marker of preeclampsia, and it reflects the severity of preeclampsia. There was significant negative correlation between flow-mediated vasodilation and plasma fibronectin, with a correlation coefficient of 0.73. This finding illustrates that we can indirectly assess endothelial function with noninvasive measurement of flow-mediated vasodilation.

Previous studies in nonpregnant subjects reported that chronic hypertension impairs vascular endothelial function. In pregnant women, however, Taylor et al reported that high plasma fibronectin levels correlate with the severity of preeclampsia and were not simply due to hypertension per se. In the current study, pregnant women with chronic hypertension showed significantly less vasodilation than normal pregnant women but significantly more vasodilation than preeclamptic women. The plasma fibronectin level in preeclampsia was significantly lower than that in normal pregnant women or chronic hypertensive pregnant women. There was a significant negative correlation between vasodilation and the plasma fibronectin level. These findings indicate that the decreased vasodilation response in preeclamptic women is probably related to endothelial dysfunction and that we can assess endothelial damage in preeclamptic women with noninvasive measurement of flow-mediated vasodilation.

Conclusion
We evaluated endothelial function during pregnancy with a 30-MHz ultrasound transducer, which provided clear visualization and accurate measurement of the radial artery was in all cases. Flow-mediated vasodilation in preeclamptic women was significantly less than in normal pregnant women or chronic hypertensive pregnant women. There was a significant negative correlation between vasodilation and the plasma fibronectin level. These findings indicate that the decreased vasodilation response in preeclamptic women is probably related to endothelial dysfunction and that we can assess endothelial damage in preeclamptic women with noninvasive measurement of flow-mediated vasodilation.

References

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