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ORIGINAL ARTICLE

Naci K. Kuscu · Ziya Kurhan · Yasemin Yildirim
Talat Tavli · Faik Koyuncu

Detection of endothelial dysfunction in preeclamptic patients by using color Doppler sonography

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Abstract Our goal in this study was to detect endothelial function in normal and preeclamptic patients by using color Doppler sonography and plasma fibronectin levels. The increased ratio of the brachial artery diameter during shear stress, and plasma fibronectin levels were measured in 15 preeclamptic and 11 normal, gestational-age matched pregnant patients. The test was repeated at the postpartum second and sixth weeks in the preeclamptic group. In addition, the plasma fibronectin levels of all patients were measured. The increased ratios were 4.26±0.69% vs 12.18±1.97% in the preeclamptic and normal patients, respectively (P=0.003). At the second and sixth postpartum weeks, the ratios were 6.67±0.89% and 9.27±1.16% in the preeclamptic group, revealing a significant improvement in the sixth week (P=0.001). Fibronectin levels were 0.80±0.11 g/L vs 0.45±0.06 g/L in preeclamptic and normal patients (P=0.01). The correlation coefficient between the fibronectin levels and increase rate was r=-0.38 and P=0.05. We conclude that endothelial dysfunction, which is fundamental to preeclampsia, can be detected by using color Doppler sonography.

Keywords Preeclampsia · Shear stress · Endothelial dysfunction

Introduction

Vascular endothelium is around 1.5 kg in a healthy adult [1] and its surface is continuously exposed to humoral factors, inflammatory mediators and changes in shear stress (SS). The function of the endothelium is to secrete vasoactive substances that regulate the vascular wall tonus and thrombus resistance according to the hemodynamic and humoral changes. The basal tonus of arteries and arterioles depend on the continuous secretion of nitric oxide (NO) from endothelial cells. Nitric oxide, which is derived from trophoblast, helps control the vascular tonus of the placenta. Less nitric oxide synthetase (NOS) activity has been detected in the placental villi of women who had preeclampsia or growth-restricted fetuses when compared to normal pregnant women [17]. In addition, higher mRNA expression of eNOS was found in both myometrium and placentae of preeclamptic women, which might be a compensatory response to an impaired vasodilatation in the uteroplacental circulation of preeclamptic patients [11]. Cardiovascular changes of pregnancy, which prevent blood pressure rise, depend on vasodilatory prostaglandins secreted by intact endothelium and NO induced by oestrogen [22]. Vascular endothelium is diffusely injured and loses its function [12, 19, 21], and plasma fibronectin levels rise in preeclampsia [3].

The goal of our study was to detect the endothelial dysfunction, which is accepted to be fundamental to preeclampsia, by measuring the dilatation in brachial artery diameter (BAD) before and after SS by using a noninvasive method that was first developed by Celermajer [5] and by measuring plasma fibronectin levels. According to Celermajer, healthy and intact endothelium with normal function can increase the diameter by more than 10%, while in an endothelium with lost function, this increase rate is below 10%.

N.K. Kuscu (✉) · Y. Yildirim · F. Koyuncu
Celal Bayar University, School of Medicine,
Department of Obstetrics and Gynecology, Manisa, Turkey 45020
e-mail: kemal.kuscu@bayar.edu.tr
Tel.: +90-236-2349070/324, Fax: +90-236-2376449

Z. Kurhan · T. Tavli
Celal Bayar University, School of Medicine,
Department of Cardiology, Manisa, Turkey

Materials and methods

Patients

15 preeclamptic (group P) and 11 gestational-age matched, normal pregnant women (group C) were enrolled in the study. Age, gestational age and clinical parameters of the patients are seen in Table 1. The study was approved by the local ethical committee of

Table 1 Demographic data of the patients

	Group P (n=15)	Group C (n=11)
Age (years)	28.1±1.5	25.1±0.6
Gestational age (weeks)	33.9±1.4	36.3±0.6
Systolic blood pressure (mmHg)	160.0±4.4 [a]	114.5±3.7 [a]
Diastolic blood pressure (mmHg)	98.3±2.4 [a]	73.6±2.0 [a]
Parity	1.9±0.4	1.3±0.15

All values are mean±SEM
[a] P<0.0001 between groups

the university hospital and the patients were told in detail about the procedure and written informed consents were obtained. Preeclampsia was diagnosed when the blood pressure was above 140/90 mmHg and proteinuria was (++) or more in two dipstick measurements at least 6 h apart. Blood was withdrawn from each patient before measurement, plasma was separated and fibronectin levels were measured by using nephelometry kit (Dade Behring, Marburg, Germany). Intra-assay and inter-assay coefficients of variations were 1.8% and 1.5%, respectively.

Doppler sonography

Endothelial function was determined by measuring BAD before and after SS by using color doppler sonography (Hewlett-Packard Image-Point 7.5 Mhz), according to the suggestions by Celermajer. Only one observer, blinded to the diagnosis of the patients at the initial measurement, completed the study. Three serial measurements were done to group P patients, the first was just after the diagnosis, the second was at the postpartum second week and the final was at the postpartum sixth week. Group C patients were measured only once during gestation. All patients had a rest in the left lateral tilt position for 10 min before the measurement. The distance between the adventitial and medial layers of the brachial artery was measured with color Doppler. Each measurement was done three times and the mean value was calculated. Then the pneumatic cuff placed on the upper arm was inflated until 250–300 mmHg and, after waiting for 3 min, the cuff was deflated and the measurements were done at 15, 30, and 60 s and two and three min. Each measurement was done only once. The mean vessel diameter and percent dilatation of each patient were obtained by averaging all measurements.

Statistical analysis

Student's t test was used to compare demographic data, Mann-Whitney-U test was used to compare the fibronectin levels and increase rate between preeclamptic and control groups, ANOVA was used to compare increase ratios at three different times within the preeclamptic group, and Pearson's correlation test was used for correlation between increase rate and fibronectin levels, P<0.05 was considered significant. Bartlett's test for homogeneity of variances was F=2.82, and ANOVA was used even though the sample size was less than 15.

Results

Color Doppler measurements are seen in Table 2, and the increase rates of both groups during the first measurement are seen in Fig. 1. The initial measurements were significantly different between group P and C (P=0.003). In the control visits of group P at postpartum second and sixth weeks, the ratios rose, but reached a significant difference only at the sixth week (P=0.001).

In groups P and C, 100% and 36% of initial measurements were below 10%, respectively. The increase rates

Table 2 Changes in brachial artery diameter before and after shear stress. *PP* postpartum; *BAD* brachial artery diameter, in millimeters; *SS* shear stress

	BAD (Before SS)	BAD (After SS)	Increase Rate (%)
Group P			
Initial	0.41±0.04	0.43±0.04	4.26±0.69 [a,b]
PP week 2	0.40±0.03	0.43±0.04	6.67±0.89
PP week 6	0.38±0.05	0.41±0.05	9.27±1.16 [a]
Group C	0.41±0.05	0.46±0.05	12.18±1.97 [b]

All values are mean±SEM
[a] P=0.001 Comparison of the first and third measurements within group P
[b] P=0.003 Comparison of the first measurement of group P with group C

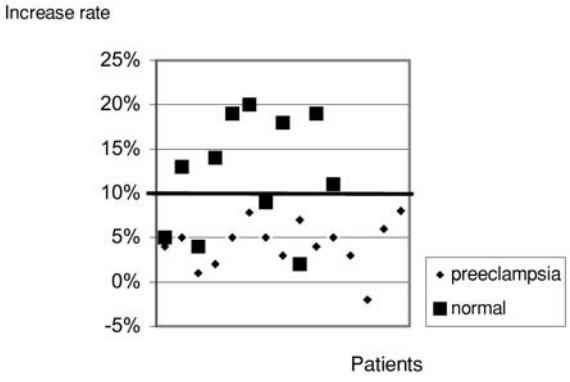

Fig. 1 Increase rates of preeclamptic and normal patients in BAD at the initial measurement.

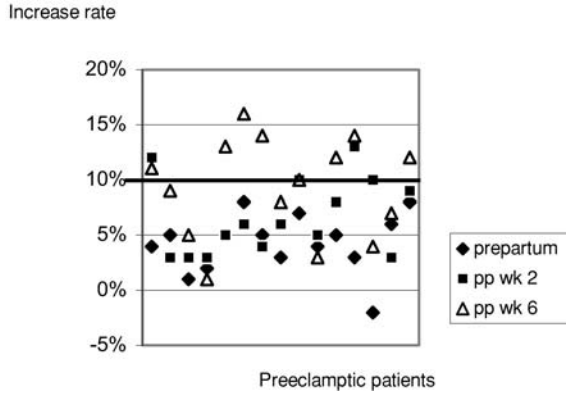

Fig. 2 Increase rates of preeclamptic patients in BAD at 3 different measurements. *Pp wk 2* postpartum week 2; *Pp wk 6* postpartum week 6

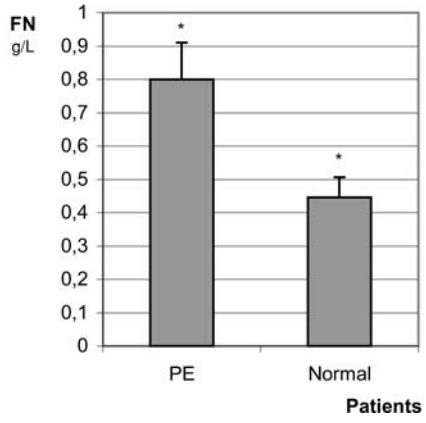

Fig. 3 Fibronectin levels of patients. *FN* fibronectin; *PE* Preeclampsia; * *P*=0.01

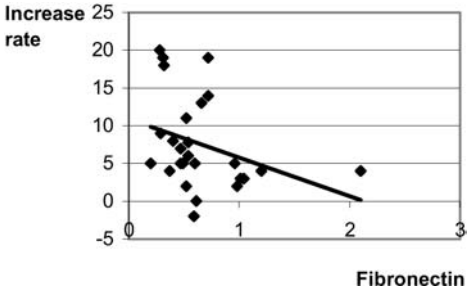

Fig. 4 Correlation of fibronectin levels with increase in BAD to SS

of group P at 3 different measurements are seen in Fig. 2. By the end of the postpartum second and sixth weeks, 18% and 73% of the measurements rose above 10%.

The plasma fibronectin levels in groups P and C were 0.80±0.11 g/L and 0.45±0.06 g/L, respectively, and are shown in Fig. 3. The difference between the two groups was significant (*P*=0.01). The correlation coefficient between the fibronectin levels and increase rate was *r*=-0.38 and *P*=0.05, revealing a moderate inverse correlation; namely, the higher the fibronectin levels, the less the increase rate of BAD to SS (Fig. 4).

Discussion

Various explanations have been forwarded in the pathogenesis of endothelial dysfunction, which is accepted to be fundamental to preeclampsia. The inhibition of endothelial cell growth by preeclamptic syncytiotrophoblasts [20], neutrophil activation and the secretion of some substances from decidual neutrophils [4, 14], endothelial and placental dysfunction due to oxidative stress [8] are within the current concepts. Preeclampsia begins with the loss of refractoriness to vasoactive agents. The major reason for this loss is the functional imbalance between the vasodilator and the vasoconstrictor substances. L-arginine, the precursor of NO, and NO levels are reduced in the umbilical vessels of these patients [18], and the main reason for this reduction is endothelial cell injury. The role of NO in the pathogenesis has been emphasized and has become much more prominent than prostacyclin (PGI_2) [6, 15]. Lower PGI_2 synthesis, reduced bioavailability of NO, an increase in cellular permeability, an increase in cell adhesion molecules in endothelial cells and prothrombotic factors all contribute to the pathogenesis of preeclampsia, and all of these changes reveal endothelial cell activation [9]. In addition, significantly reduced mRNA expression of endothelin receptor was reported in the myometrium and placentae of preeclamptic women, which might represent down regulation of the receptors due to the increased levels of endothelin-1 in uteroplacental circulation in this disorder [10].

Endothelial injury and resultant endothelial dysfunction have so far been shown by elevated biochemical markers in the plasma [16]. In our study, we measured endothelial function by a non-invasive SS test and the increase rate of all preeclamptic patients was below 10% (the highest ratio was 7.8%). This method, which has been widely used for cardiovascular patients, can also be used to detect the endothelial function in obstetric practice. Impaired function in preeclamptic patients might be due to less NO release from the cells. The function did not reach normal level at postpartum second week, but was almost normal at the sixth week, which showed that endothelial function resumed by the end of puerperium. The patients regained the ability to dilate their vessels against hypoxia caused by SS.

Grimfors et al. investigated endothelial function in preeclamptic, normal pregnant and non-pregnant women with acetylcholine and sodium nitroprusside iontophoresis, and found the same degree of vasodilation in all groups [13]. They concluded that preeclampsia, probably, did not cause endothelial cell injury severe enough to be detected with this method and microvascular endothelial function was not impaired in mild cases. But we found a significant difference between preeclamptic and normal patients at the initial measurement, and this difference did not completely resolve by the second postpartum week.

In another study, flow-induced relaxation of the arteries was found to be highest in normal pregnant women when compared with non-pregnant controls and preeclamptic women [7]. When the normal pregnant women were given a NOS inhibitor, relaxation of the arteries decreased. The authors emphasized the role of NO in preeclampsia, and failure of flow-induced dilatation was considered to play a role in the pathogenesis of the disorder.

In an in vitro study, endothelial cells exposed to preeclamptic serum, increased NO secretion more than the cells that were exposed to normal pregnant serum [2]. But when SS was applied, though, the cells with normal serum could increase NO secretion, the other group could not show the same increase. The authors conclud-

ed that some factors in preeclamptic serum induced the cells to work with maximum capacity and SS could not make an additional stimulus to secrete more NO.

The plasma fibronectin level, a biochemical marker of endothelial cell injury, had a moderate negative correlation with increased rates in BAD. Patients with higher fibronectin levels could not dilate their vessels against SS, as well as the controls. But, in the control group, four patients also had lower increase rates that might limit the general use of this method as a screening tool due to probable low specificity. We did not perform sensitivity and specificity tests because of our limited sample size. If the test is reproduced with a higher number of patients, it may be possible to have better results. Smoking, high fibrinogen level and hypercholesterolemia preclude the vessels to dilate to the normal level. As physiological changes of pregnancy cause elevated levels of fibrinogen and cholesterol, these factors might have obscured the expected result of all of our patients in group C.

In conclusion, color Doppler sonography may be used to detect endothelial dysfunction in preeclamptic women and this may help to search the different vascular dynamics seen in hypertensive disorders of pregnancy. In addition, this non-invasive test may be used as a screening method alone or in combination with an endothelial marker in pregnant women without any hypertensive problems to find out endothelial dysfunction time and this may predict oncoming preeclampsia as endothelial dysfunction may precede the symptoms of the disorder.

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