Elevated plasma endothelial microparticles in preeclampsia

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OBJECTIVE: The purpose of this study was to assess endothelial dysfunction in women with preeclampsia by measuring endothelial microparticles.

STUDY DESIGN: A case-control study was conducted on 20 women with preeclampsia and 20 healthy pregnant women as control subjects. Endothelial microparticles were measured by flow cytometry with anti-CD31 and anti-CD42. $CD31^+/CD42^+$ platelet microparticles were also quantified.

RESULTS: Plasma endothelial microparticles levels were elevated significantly in women with preeclampsia as compared with control subjects (mean ± SD and median [range]: 14,723 ± 7,724 counts/μL and 12,378 counts/μL [1,442-33,772 counts/μL] vs 8406 ± 2832 counts/μL and 9016 counts/μL [3,381-12,806 counts/μL]; $P < .001$). Plasma platelet microparticles levels were not different among cases compared to control subjects (10,751 ± 6,114 counts/μL and 9463 counts/μL [3,000-23,895 counts/μL] vs 7871 ± 4344 counts/μL and 6462 counts/μL [444-18,947 counts/μL]; $P = .208$). No significant correlation was found between plasma endothelial microparticles and mean arterial pressure in cases or control subjects.

CONCLUSION: The elevation of endothelial microparticles in women with preeclampsia supports the endothelial injury theory in preeclampsia. (Am J Obstet Gynecol 2003;189:589-93.)

Key words: Endothelial microparticle, preeclampsia, endothelial injury

Preeclampsia is a pregnancy-associated disorder of unknown cause. Many theories have been proposed, including, but not limited to, abnormal trophoblast invasion, coagulation abnormalities, immunologic phenomena, genetic predisposition, dietary abnormalities, and vascular endothelial damage.[1] Important evidence exists to implicate endothelial cell injury to this pregnancy-specific disease. The pathologic changes that are associated with preeclampsia suggest that generalized maternal vascular dysfunction could explain the vasospasm, edema, proteinuria, coagulopathy, and renal and hepatic abnormalities that are hallmarks of the disease.[2] Levels of fibronectin, vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1, E-selectin, CD31, and von Willebrand factor have been documented to be elevated in patients with preeclampsia even before its clinical manifestation.[3-6] Endothelial cells shed vesicles into the circulation on activation or apoptosis. These vesicles, termed *endothelial microparticles* (EMPs), contain cytoplasmic components and negatively charged phospholipids bearing some of the cell's surface proteins.[7] Our laboratory has developed flow cytometric methods for the detection of EMPs as an indicator of endothelial injury. In vitro and in vivo, EMPs have been shown to express CD31 but not CD42.[7] The absence of the latter marker distinguishes EMPs from platelet microparticles (PMP), which are released on platelet activation and express both antigens. Elevated EMPs have been shown in thrombotic and immunologic disorders such as thrombotic thrombocytopenia purpura (TTP),[7] multiple sclerosis,[8] and coronary heart disease.[9,10] The objective of this study was to assess endothelial dysfunction in patients with preeclampsia by measuring $CD31^+/CD42^-$ EMPs.

Methods

Patient and control population. This prospective, case-control study was conducted with 20 women with preeclampsia and 20 healthy pregnant women at the University of Miami/Jackson Memorial Hospital from August 2000 to July 2001. The criteria for the selection of cases were defined by the Working Group Report on High Blood Pressure in Pregnancy, as follows: blood pressure of ≥140 mm Hg systolic or ≥90 mm Hg diastolic that occurs after 20 weeks of gestation in a woman with previously normal blood pressure and proteinuria that was defined as urinary excretion of ≥0.3 g protein in a 24-hour period.[11] Patients in labor and those patients with chronic

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Table. Overall and group-specific demographic, anthropometric, and clinical characteristics of study participants

Characteristic	*Overall*	*Control*	*Preeclampsia*	*P value**
Age (y)	27.4 ± 6.0	27.3 ± 5.0	27.5 ± 7.0	.918
Estimated gestational age (wk)	37.6 ± 2.4	38.4 ± 2.1	36.8 ± 2.4	.031
Body mass index (kg/m^2)	31.9 ± 5.9	28.9 ± 4.3	34.9 ± 5.8	<.001
Birth weight (g)	3129.8 ± 695.0	3312.8 ± 593.9	2946.9 ± 753.9	.096
MAP (mm Hg)	97.2 ± 18.1	81.2 ± 9.5	113.3 ± 6.6	<.001
Parity†	0 (0-3)	0 (0-3)	0 (0-2)	.136

Data are given as mean ± SD.
*All are two-tailed probability values and correspond to two independent sample *t* tests, except for parity, which corresponds to a Mann-Whitney test.
†Expressed as median (range).

hypertension, pregestational diabetes mellitus, and renal or hepatic disease were excluded. Control subjects were available from healthy pregnant women of similar demographic characteristics (Table). Approval from the University of Miami School of Medicine Institutional Review Board was obtained. Mean arterial pressure (MAP) was calculated in all patients with the use of blood pressure readings before blood drawing. Body mass index was calculated in all patients, and the information on birth weight in all neonates was available for comparison between the two groups. Levels of proteinuria and platelet counts were also collected in all women with preeclampsia.

Preparation of samples for EMP and PMP assay. Blood samples were collected in sterile tubes that contained sodium citrate before intravenous hydration, magnesium sulfate infusion, or antihypertensive medications. A flow cytometric assay of EMP and PMP with the use of fluorescent monoclonal antibodies anti-CD31 and anti-CD42 was used, as previously described.[7] Plasma samples were double labeled with CD31/CD42 to exclude CD31$^+$ microparticles of platelet origin. CD31$^+$/CD45$^+$ particles account for a negligible percentage of all CD31$^+$ microparticles, which would represent a subpopulation of leukocyte microparticles and therefore would not have a significant impact on EMP counts.[7,8] To rule out possible contamination with leukocyte microparticles, we conducted a pilot study using a small number of samples. In this pilot study, we added anti-CD45 to the assays with anti-CD31. The results indicate that, in CD31$^+$ microparticles, only a small fraction (<5%) is coexpressed with panleukocyte marker CD45. We concluded that CD31$^+$/CD42$^-$-labeled microparticles are derived from endothelium. Briefly, as previously described,[7,8] fresh platelet-poor plasma was prepared and assayed within 4 hours of venipuncture to avoid contamination with microparticles that were released ex vivo. Fifty microliters of platelet-poor plasma was incubated with 4 μL of phycoerythrin anti-CD31 and 4 μL of fluorescein isothiocyanate-conjugated anti-CD42. Samples were incubated for 15 minutes and then diluted with 0.5 mL of phosphate-buffered saline solution. EMPs are defined as all particles that are positive for CD31 and negative for CD42; PMPs are considered to be all particles coexpressing both antigens (CD31$^+$/CD42$^+$). Counts for particles were triggered by fluorescence 2 above a preset threshold on a Coulter EPICS XL flow cytometer (Beckman Coulter, Miami, Fla; Fig 1). Daily calibration with fluorescent beads (Beckman Coulter) ensured that fluctuations were <2%. Isotype-matched control values for each fluorophore were subtracted from EMP counts.

Statistical analysis. Two independent sample *t* tests were used to compare cases versus control subjects with respect to continuous variables with normal or approximately normal distributions, such as maternal age, estimated gestational age, and birth weight of the neonates. The Mann-Whitney test was used to compare the two study groups with respect to variables with skewed distributions, such as EMP and PMP levels. Also because of the skewness of the distributions, the associations between EMPs and PMPs with proteinuria and platelet counts were assessed with the use of Spearman rank correlations. Tests with corresponding two-sided probability values of <.05 were considered statistically significant.

Results

As shown in the Table, there were no statistically significant differences between cases and control subjects with respect to age, parity, and child's birth weight, although women with preeclampsia tended to be delivered of babies with lower birth weights. The cases were significantly more obese and were delivered at an earlier gestational age. All infants were found to be appropriate for gestational age with respect to birth weight. There were three cases of severe preeclampsia that met the inclusion criteria. There were no smokers. As anticipated by the diagnostic criteria that was used to recruit women with preeclampsia, the MAP was higher in these women. There were no study participants with thrombocytopenia (<100,000/μL), and the platelet count ranged from 150,000 to 650,000/μL, with a median of 219,500/μL. The mean ± SD protein concentration in 24-hour urine collections among women with preeclampsia was 1,893.4 ± 3,837.1 mg, with a range of 315 to 17,138 mg

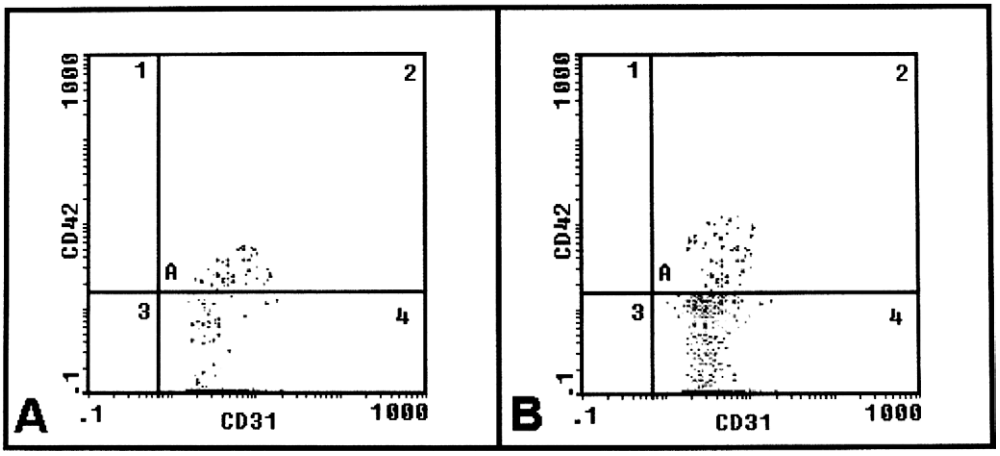

Fig 1. Representative printout of flow cytometric determination of $CD31^+/CD42^-$ EMP and $CD31^+/CD42^+$ PMP in normal pregnant control subjects **(A)** and patients with preeclampsia **(B)**. Region 4 (*lower right*) is $CD31^+/CD42^-$, which represents EMP, whereas region 2 (*top right*) is $CD31^+/CD42^+$, which represents PMP. EMPs express only CD31, whereas PMPs coexpress CD31 and CD42. As can be seen, $CD31^+/CD42^-$ EMP were elevated significantly in the representative preeclamptic example. On the other hand, no significant difference in PMPs was observed between control subjects and women with preeclampsia.

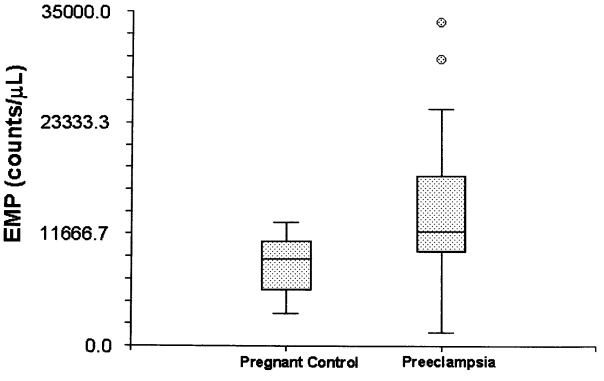

Fig 2. EMP (counts per microliter) in normal pregnant control subjects and women with preeclampsia. The *box plot* shows a significant elevation in the levels of $CD31^+/CD42^-$ EMP in women with preeclampsia compared with control subjects ($P < .01$).

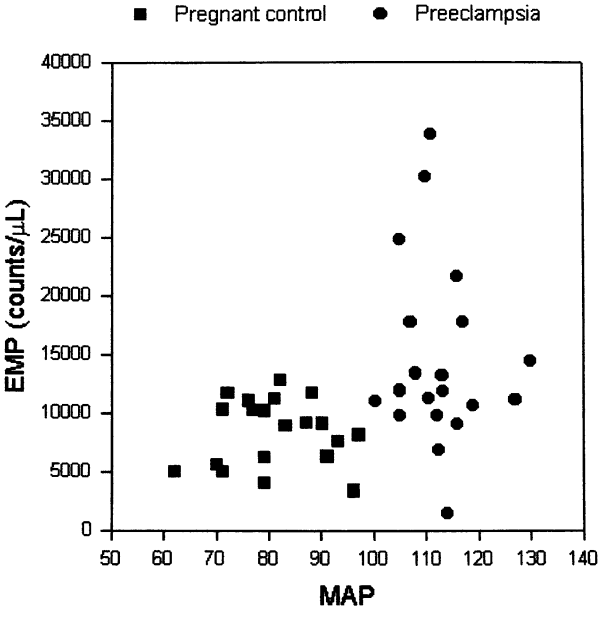

Fig 3. Correlation between EMP and MAP in normal pregnant control subjects and women with preeclampsia. No association was found between EMP counts and the severity of hypertension in both patient groups. The *number on the x axis* represents MAP (millimeters of mercury); the *number on the y axis* represents EMPs (counts per microliter).

and a median of 547.5 mg. The plasma EMP assay revealed significantly elevated levels (counts per microliter) in the women with preeclampsia as compared with the control group (14,723 ± 7,724 counts/μL, 12,378 counts/μL [range, 1,442-33,772 counts/μL] vs 8,406 ± 2,832 counts/μL, 9,016 counts/μL [3,381-12,806 counts/μL]; Mann-Whitney test, $P < .001$; Fig 2). In contrast, although elevated, $CD31^+/CD42b^+$ PMP levels (counts per microliter) were not significantly different in the women with preeclampsia compared with healthy pregnant control subjects (10,751 ± 6,114 counts/μL and 9,463 counts/μL [3,000-23,895 counts/μL] vs 7,871 ± 4,344 counts/μL and 6,462 counts/μL [444-18,947 counts/μL]; Mann-Whitney test, $P = .208$). However, because of the small number of patients who were enrolled in this study, the statistical power of this test is 39%; therefore, whether this subset of PMPs is elevated in preeclampsia remains inconclusive. There was no association between plasma EMP levels and the MAP among either cases (Spearman rank correlation, −0.23; $P = .318$) or control subjects (Spearman rank correlation, −0.01; $P = .967$; Fig 3). Similarly, among women with preeclampsia, there was no significant association between proteinuria and EMPs

(Spearman rank correlation, 0.11; $P = .653$) or platelet count and PMPs (Spearman rank correlation, 0.43; $P = .058$). However, the lack of significance of this latter correlation may be due to the small sample size (n = 20) and the corresponding low statistical power of 49%.

Comment

Endothelial dysfunction or injury has been implicated as a pivotal player in the pathophysiologic condition of preeclampsia on the basis of histologic findings and elevated soluble markers of endothelial cell injury.[12] However, no direct measurement of endothelial cell damage has been reported. The current study documents the elevation of $CD31^+/CD42^-$ EMPs in patients with preeclampsia. We have reported previously that $CD31^+/CD42^-$ EMPs are elevated in TTP,[7] multiple sclerosis,[8] and coronary artery disease,[9,10] which implicates endothelial injury in these disorders. The EMP counts increased during the active phase of TTP and multiple sclerosis and decreased on remission. Endothelial injury in TTP promotes platelet activation that leads to the formation of platelet-rich thrombi in small vessels. The platelet-rich thrombi in the microcirculation lead to severe thrombocytopenia, microangiopathic hemolytic anemia, and transient central nervous system dysfunction.[13] Similarly, thrombocytopenia, platelet activation, and hemolytic anemia also develop in preeclampsia.

The factors that are responsible for the release of EMPs in preeclampsia remain to be elucidated. Possible candidates include tumor necrosis-α and interleukin-1β, which have been shown to perturb endothelial cells in vitro.[14,15] It is also possible that multiple factors may work in synergy to injure the endothelium and promote the release of EMPs.

This investigation was designed to compare the plasma concentration of EMPs among normal pregnant patients and patients with the diagnosis of preeclampsia. Although EMPs correlated well with the clinical presentation, no significant correlation was observed between $CD31^+/CD42^+$ PMPs and preeclampsia in the patients who were studied. Further measurement of $CD31^+/CD42^+$ PMPs in larger patient populations are required to clarify the presence of PMPs in preeclampsia. Because of the limited sample size of this study, the power to determine whether PMPs are significantly elevated in preeclampsia is only 39%.

The measurement of plasma EMPs is emerging as a useful marker of endothelial injury. EMPs are assayed readily in small amounts of peripheral blood samples and provide useful information on the status of the endothelium. This study, however, only included women with preeclampsia that was diagnosed in the third trimester. The next step is to study women from early in gestation and follow them with serial blood sampling to determine if EMPs are elevated before the clinical development of preeclampsia. Such a study would also allow us to determine whether an elevation in EMP levels would recognize women who have preeclampsia at earlier gestational age or a more severe form of the disease. Quantification of EMPs may be a useful objective tool in the conservative treatment of severe preeclampsia that is remote from term, if progressively increasing levels denote worsening disease in a patient who is clinically stable. Furthermore, because we did not find an association between the elevation of EMP level and the severity of hypertension, this marker might be useful in distinguishing between gestational hypertension and preeclampsia.

In conclusion, the significant elevation in EMPs in this study supports the theory of endothelial injury in the pathogenesis of preeclampsia. Further studies of EMPs are needed to evaluate their diagnostic and prognostic value in preeclampsia.

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