OBSTETRICS

Urinary podocyte excretion as a marker for preeclampsia

Vesna D. Garovic, MD; Steven J. Wagner, MD; Stephen T. Turner, MD; David W. Rosenthal, MD; William J. Watson, MD; Brian C. Brost, MD; Carl H. Rose, MD; Larisa Gavrilova, MD; Paula Craigo, MD; Kent R. Bailey, PhD; Johannes Achenbach, MS V; Mario Schiffer, MD; Joseph P. Grande, MD, PhD

OBJECTIVE: The objective of this study was to examine whether podocyturia, which is the urinary excretion of viable podocytes (glomerular epithelial cells), is present in urinary sediments of patients with preeclampsia. We also aimed to compare the test characteristics of podocyturia to those angiogenic factors that have been shown to play an important role in the pathogenesis of preeclampsia (s-Flt-1, PlGF, and endoglin).

STUDY DESIGN: Serum angiogenic factors were measured in 44 patients with preeclampsia and 23 normotensive control patients. In a patient subset (15 cases and 16 control patients), urinary proteinuria were identified and quantified on the basis of their expressions of podocyte-specific proteins.

RESULTS: Urinary podocyte excretion occurred in all patients with preeclampsia. The positive predictive value for the diagnosis of preeclampsia was greater for podocyturia than for any of the measured angiogenic factors.

CONCLUSION: Podocyturia is a highly sensitive and specific marker for preeclampsia. It may contribute to the development of proteinuria in preeclampsia.

Key words: podocyturia, podocyte, preeclampsia

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Preeclampsia is a pregnancy-specific disease that affects approximately 5% of all pregnancies and remains a leading cause of both maternal and fetal morbidity and death worldwide.[1] It is characterized by hypertension (blood pressure, ≥140/90 mm Hg) and proteinuria (≥300 mg in a 24-hour urine sample) that occur after 20 weeks of gestation.

Proteinuria in preeclampsia is associated with characteristic renal pathologic changes of glomerular endotheliosis, which is considered to be a hallmark of preeclampsia in humans, but its underlying mechanisms are poorly understood. The role of podocyte (glomerular epithelial cell) damage and ultimate loss in the development of proteinuria has been supported by experimental and clinical studies of patients with glomerular diseases. Podocyte loss can occur as the result of either apoptosis[2,3] or urinary excretion that is subsequent to podocyte detachment from the glomerular basement membrane. Published studies have shown that viable podocytes are present in the urine of patients with a variety of glomerular diseases that are associated with proteinuria and have indicated that podocyturia seems to be confined to active disease only, in contrast to proteinuria, which is present during both active and chronic phases of glomerular damage.[4,5] Given the acuteness of kidney injury in preeclampsia, we postulated that urinary podocyte loss occurs concurrently with proteinuria and correlates with its severity. The first 2 aims of this study were (1) to test the hypothesis that urinary excretion of viable podocytes, which were identified and quantified on the basis of the expressions of podocyte-specific proteins (podocalyxin, podocin, nephrin, and synaptopodin), is present in the urine samples of pregnant women with clinically confirmed preeclampsia and (2) to correlate urine podocyte counts with the degree of proteinuria.

In addition, we aimed to compare the test characteristics between podocyturia and angiogenic factors that have been shown to play an important role in the pathogenesis of preeclampsia. Several lines of evidence have suggested that preeclampsia is associated with elevated levels of the soluble receptor for vascular endothelial growth factor, commonly referred to as fms-like tyrosine kinase receptor-1 (sFlt-1), which may bind, neutralize, and decrease free levels of vascular endothelial growth factor and placental growth factor (PlGF), both of which are required for active fetal and placental angiogenesis in pregnancy. More recently, elevated levels of circulating soluble endoglin were reported to interfere with TGF-β1 signaling and nitric

From the Divisions of Nephrology and Hypertension (Drs Garovic, Wagner, Turner, Rosenthal, and Grande) and Obstetrics and Gynecology (Drs Watson, Brost, Rose, and Gavrilova) and the Departments of Anesthesia (Dr Craigo) and Biostatistics (Dr Bailey), Mayo Clinic, Rochester, MN, and the Department of Nephrology, Hannover Medical School, Hannover, Germany (Dr Schiffer and Mr Achenbach).

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Reprints: Vesna D. Garovic, M.D, Division of Nephrology and Hypertension, Mayo Clinic, 200 First St SW, Rochester, Minnesota 55905; garovic.vesna@mayo.edu.

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TABLE 1
Patient characteristics

Variable	Normal (n = 23)	Preeclampsia (n = 33)	HELLP (n = 11)	Preeclampsia + HELLP (n = 44)
Maternal age (y)	28.7 ± 5.4	26.2 ± 5.1	33.0 ± 6.0	27.9 ± 6.1
Gestational age (wk)	39.2 ± 2.2	34.3 ± 3.8*	33.5 ± 5.6*	34.1 ± 4.2*
Primiparous (%)	47.8	81.8	9.1	63.6
Systolic blood pressure (mm Hg)	110.5 ± 9.5	159 ± 19.8*	162.6 ± 23*	159.9 ± 20.3*
Diastolic blood pressure (mm Hg)	66.9 ± 9.8	97.8 ± 9.4*	98.3 ± 10.6*	97.9 ± 9.6*
Proteinuria (g/24 hr)	247 ± 294	2693 ± 3164*	4373 ± 5962*	3113 ± 4032*
Platelet count	242,000 ± 35,519	232,333 ± 66,787	100,273 ± 42,245*	199,318 ± 84,146

Data are given as mean ± SD.

* $P < .05$, compared with normal group.

oxide–mediated vasodilation. Our third aim was to compare the test characteristics of podocyturia, sFlt-1, PlGF, and endoglin in patients with a clinically confirmed diagnosis of preeclampsia.

Materials and Methods

This study was approved by the Institutional Review Board, and all women were consented before inclusion in the study. The diagnosis of preeclampsia was made in the presence of (a) hypertension after 20 weeks of gestation, which was defined as a blood pressure of ≥140/90 mm Hg, (b) proteinuria, which was defined as ≥300 mg of protein in a 24-hour urine specimen, and/or 1+ (30 mg/L) dipstick urinalysis in the absence of urinary tract infection and/or a predicted 24-hour urine protein of >300 mg on a random urine collection, and (c) resolution of hypertension and proteinuria by 12 weeks after delivery. We also included women with severe forms of preeclampsia such as eclampsia and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, the diagnosis of which was confirmed on the basis of previously published criteria.[6] Healthy, normotensive pregnant women without hypertension and proteinuria served as control subjects. An additional control group consisted of women with hypertension and proteinuria.

A cross-sectional study was conducted, and blood and urine samples were collected close to and typically ≤24 hours before delivery. In total, 67 women were recruited. Preeclampsia was present in 33 of the patients, and HELLP was diagnosed in 11 patients; 23 normotensive pregnancies served as control subjects (Table 1). Blood samples were obtained in all 67 women, and urine samples for podocyturia were collected in a subset of 31 pregnant women (15 cases and 16 control subjects).

Serum studies

Blood samples for the determination of sFlt-1, free PlGF, and soluble endoglin levels were drawn within 24 hours before delivery. Serum creatinine level, liver function tests, and platelet counts were performed according to standardized laboratory procedures. Serum levels of sFlt-1, soluble endoglin, and free PlGF were measured with Quantikine ELISA (enzyme-linked immunosorbent assay) kits (R&D Systems, Minneapolis, MN).

Urine chemistry

Concurrent with serum collection, clean-catch urine specimens (50-100 mL) were obtained. Urine albumin, total protein, and creatinine concentrations were measured by standard methods on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Urinary PlGF determinations were performed with the PlGF ELISA (enzyme-linked immunosorbent assay) kit (R&D Systems).

Podocyturia

Random urine samples (25-50 mL each) were centrifuged for 8 minutes at 700*g* at room temperature. The pellets were rinsed twice with human diploid fibroblast (HDF) solution. Next, the pellets were resuspended in Dulbecco's modified eagle's medium (DMEM) F-12 medium with 10% fetal bovine serum that was supplemented with antibiotics for the prevention of bacterial contamination. One-milliliter aliquots were plated in 4-chamber, collagen-coated tissue culture slides, which was followed by overnight incubation at 37°C in 5% CO_2. The next day, the media were removed, followed by 2 phosphate-buffered saline solution washes. Slides were fixed with 1 mL of ice cold methanol for 10 minutes at −20°C. Each of the 4 slide chambers was incubated with 1 of 4 different antibodies to podocyte proteins: podocalyxin (dilution, 1:40), podocin (dilution, 1:200), nephrin (dilution, 1:100), and synaptopodin (undiluted). After being washed with phosphate-buffered saline solution, a secondary fluorescein isothiocyanate–labeled antibody was added at a dilution of 1:40 for 30 minutes. The sediment was counterstained with Hoechst nuclear stain to facilitate differentiation of whole cells from cell fragments. Coverslips were mounted with Vectashield (Vector Labs, Burlington, CA), and the slides were viewed with a fluorescence microscope (Leica, Germany). Nucleated, positive-staining cells were considered to be podocytes. A renal pathologist (J.P.G.), who was blinded to the clinical diagnosis and laboratory findings, evaluated each sample to determine the number of cells that were