RESEARCH ARTICLE | Hypertensive Disorders of Pregnancy: Effects on Mother and Baby

Longitudinal characterization of renal proximal tubular markers in normotensive and preeclamptic pregnancies

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Codiš E, Garovic VD, Gonzalez-Suarez ML, Milic N, Borowski KS, Rose CH, Davies NP, Kashani KB, Lieske JC, White WM. Longitudinal characterization of renal proximal tubular markers in normotensive and preeclamptic pregnancies. Am J Physiol Regul Integr Comp Physiol 312: R773–R778, 2017. First published April 24, 2017; doi:10.1152/ajpregu.00509.2016.—Glomerular damage is common in preeclampsia (PE), but the extent and etiology of tubular injury are not well understood. The aim of this study was to evaluate tubular injury in patients with PE and to assess whether it predates clinical disease. We performed a prospective cohort study of 315 pregnant women who provided urine samples at the end of the second trimester and at delivery. This analysis included women who developed PE (n = 15), gestational hypertension (GH; n = 14), and normotensive controls (NC; n = 44). Urinary markers of tubular injury, α1-microglobulin (A1M), retinol-binding protein (RBP), kidney-injury molecule-1 (KIM1), complement C5b-9, tissue inhibitor metalloproteinase-2 (TIMP-2), and insulin-like growth factor binding protein-7 (IGFBP-7) were measured by enzyme-linked immunosorbent assay (ELISA) and reported in relation to urine creatinine concentration. Second-trimester concentrations of all markers were similar among groups. At delivery, A1M concentrations were higher in the PE group than in the GH and NC groups (medians 9.85, 0.05, and 0.28 ng/mg, respectively, P < 0.01). Concentrations of C5b-9 were higher in the PE group than in the GH and NC groups (66.7, 8.3, and 35%, respectively, P = 0.01). Concentrations of C5b-9 were higher in the PE group than in the GH and NC groups (66.7, 8.3, and 35%, respectively, P = 0.01). Concentrations of C5b-9 were higher in the PE group than in the GH and NC groups (medians 9.85, 0.05, and 0.28 ng/mg, respectively, P = 0.003). KIM1, RBP, TIMP-2, and IGFBP-7 concentrations did not differ among groups at delivery. In conclusion, proximal tubular dysfunction, as assessed by A1M and C5b-9, developed during the interval between the end of the second trimester and delivery in patients with PE. However, this was not matched by abnormalities in markers previously associated with tubular cell injury (KIM-1, IGFBP-7, and TIMP-2).

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tubular injury; kidney disease; preeclampsia

PREECLAMPSIA (PE) is a multisystem disease classically characterized by hypertension and proteinuria that typically develops beyond 20 wk of pregnancy (1) and affects ~3–5% of pregnant women worldwide.(36A) The pathophysiology of PE is marked by a systemic inflammatory response and is divided into an initial preclinical stage that subsequently evolves into overt clinical disease (15, 40, 42). Glomerular injury, as marked by proteinuria, is the hallmark of this disease and is the primary feature that distinguishes PE from gestational hypertension (GH).

Research to date has focused on markers of glomerular injury, reporting that both glomerular endotheliosis and podocyturia (i.e., urinary loss of viable podocytes,glomerular epithelial cells) are important components of renal injury that are manifested by proteinuria in women with PE (16, 19, 20, 47, 48). Our previous study demonstrated that podocyturia precedes the development of clinical disease and, therefore, is not exclusively the result of hypertensive kidney injury (16). In contrast, only a limited number of studies in PE patients have examined tubular dysfunction and injury. These studies have revealed that tubular damage in general is also present in this patient population and that complement activation in particular is associated with proximal nephron injury in patients with severe PE (12, 38, 43, 46, 50).

Because the systemic inflammatory response is associated temporally with the clinical signs and symptoms of PE and in turn can cause tubular injury, as measured by known urinary markers, we hypothesized that tubular dysfunction is present at the time of clinical disease in addition to glomerular damage that has been demonstrated previously (16). We posited further that tubular dysfunction occurs before the development of clinical disease. The primary aim of our study was to characterize the urinary concentrations of urinary markers of tubular dysfunction or injury and complement activation in patients with PE and GH and in normotensive controls (NC) at the end of the second trimester and at the time of delivery to document the timing of tubular damage onset in PE.

MATERIALS AND METHODS

Patients were selected from a prospective cohort of 315 pregnant patients recruited between 2007 and 2011 at the Mayo Clinic (Rochester, MN), the details of which have been described previously (16, 20). Briefly, patients were enrolled in the study at their initial prenatal visits after giving informed consent and were followed throughout pregnancy until 4 to 8 wk postpartum. Urine samples were collected at 25–28 wk gestation and within 24 h before delivery. Clean/catch urine samples (50–100 ml) were collected in sterile containers and then separated into aliquots and processed as described previously.

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We selected all patients who developed PE and GH, as well as a subsample of the NC, for this study, which was approved by the Mayo Clinic Institutional Review Board. All participants signed informed consent forms at the time of enrollment. The NC were matched 3:1 for maternal age and parity with the PE cases. Pregnancy outcomes were ascertained at the time of hospitalization for delivery. Diagnoses of PE, GH, and HELLP syndrome (defined based on the presence of hemolysis, elevated liver enzymes, and low platelet count) were made according to clinical criteria from the American College of Obstetricians and Gynecologists’ Task Force on Hypertension in Pregnancy (1).

Urinary concentrations of kidney injury molecule-1 (KIM-1) (R & D Systems, Minneapolis, MN) and complement C5b-9 (BD Biosciences, San Jose, CA) were measured from samples collected previously at 25–28 wk gestation, and within 24 h of delivery, using human quantikine enzyme-linked immunosorbent assay (ELISA). α1-Microglobulin (A1M) and retinol-binding protein (RBP) were measured using a BNII nephelometer assay (Siemens, Newark, DE) in the Mayo Renal Testing Laboratory. Analysis of the samples was made after no dilution or up to a 1:100 dilution if the concentration exceeded the assay range for the particular ELISA performed. Urinary concentrations of two novel markers for acute kidney injury, tissue inhibitor metalloproteinase 2 (TIMP-2), and insulin-like growth factor binding protein 7 (IGFBP-7) were determined using the NephroCheck Assay Kit (Astute Medical) The acute kidney injury (AKI) risk score was calculated according to the following formula: TIMP2 × IGFBP7 (ng/ml)^2/1,000. The AKI risk score has been validated for the identification of acute kidney injury (26). The urinary concentrations of albumin, total protein, and creatinine were measured using standard assays on a Cobus c511 autoanalyzer in the Mayo Renal Testing Laboratory (Roche Diagnostics).

Statistics. Demographic and clinical covariates, including serum creatinine, were collected by chart review. Urinary marker concentrations were calculated as ratios compared with urinary creatinine concentrations and were reported as medians (range). Numeric variables were compared with the Kruskal-Wallis test and categorical variables with the Chi-square test. A P value of <0.05 was considered statistically significant. Data analysis was performed using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

From the cohort of 315 pregnant patients, 15 were diagnosed with PE and 15 with GH (Fig. 1). One patient diagnosed with GH did not have adequate urine sample volumes and, therefore, was excluded from the study. A total of 44 NC were selected, as one PE patient had only two possible age- and parity-matched NC in the cohort.

Demographic variables are summarized in Table 1. The NC were not significantly different from those with either GH or PE with respect to age, body mass index, or nulliparity. However, the three groups (PE, GH, and NC), as expected, differed in both gestational age (38.7, 39.3, and 39.9 wk, respectively, P = 0.007) and mean systolic blood pressure (144, 141, and 120 mmHg, respectively, P < 0.001) at delivery. There also was a higher proportion of smokers in the PE group compared with the NC group. In the PE group, six patients had severe features (4 with severe-range blood pressures ≥160/110 mmHg, 1 with elevated liver enzymes, and 1 patient with a refractory headache). Only one PE patient had an elevated serum creatinine (Cr = 1.0 mg/dl) based on the proposed normal range of 0.4–0.9 mg/dl for creatinine in the third trimester of pregnancy (Table 1) (3, 31).

Urinary concentrations of KIM1, A1M, C5b-9, and RBP, between 25 and 28 wk of gestation, were similar among all three groups (Table 2). A1M concentrations at delivery were significantly higher in the PE group compared with either the GH group or the NC both as absolute values (medians 20, 8, and 15 mg/g, respectively, P = 0.008), and as A1M-to-creatinine ratios >13 (66.7, 8.3, and 35%, respectively, P = 0.01). Complement C5b-9 concentrations were also significantly higher in the PE patients compared with the GH and NC groups (medians of 9.85, 0.05, and 0.28 ng/mg, respectively, P = 0.003). There were no statistically significant differences in the concentrations of the other markers of tubular injury (KIM1, RBP, TIMP-2, and IGFBP-7) among the groups at delivery. AKI risk scores did not differ significantly among the
groups at delivery. Interestingly, RBP concentrations remained higher than the nonpregnant reference values (<130 μg/g) at both time points in the majority of patients in the PE, GH, and NC groups (86.7, 76.9, and 90.7%, respectively, in the second trimester; 86.7, 75, and 87.5%, respectively, at delivery).

DISCUSSION

Most of the renal injury research in PE to date has focused on glomerular injury because proteinuria is typically the predominant aspect of the disease and AKI is relatively rare, even in the hypertensive pregnancy disorders (HPD), affecting only 1% of preeclamptic pregnancies (30). The infrequent diagnosis of AKI in HPD is due partly to a lack of consensus in the definition of AKI in pregnancy and the physiological pregnancy changes that have to be taken into consideration when defining AKI (4). Increased glomerular filtration rate, kidney blood flow, and solute clearance all affect serum creatinine and BUN, hence, the lower creatinine values observed in pregnant women (4, 18). Elevations in serum creatinine in patients with HPD often go unnoticed because the levels most often stay within normal nonpregnant laboratory reference values. Consequently, tubular cell function has not been studied well in this patient population, although the oxidative stress and pro-inflammatory/hypercoagulable states that characterize PE are known mediators of tubular injury.

This is the first study evaluating tubular kidney function before and at the time of clinical onset of PE. Our study extends previous findings that implicated complement activation (as demonstrated by an increase in urinary excretion of C5b-9) in the proximal tubular injury associated with severe PE. Because the majority of our PE cases (9 of 15) had mild disease, similar pathways may be implicated in all forms of PE (mild and severe) (12, 13). Of note is that PE is increasingly recognized as a heterogeneous disease, and different clinical subtypes may reflect distinct underlying pathological mechanisms (17, 49). It is common in clinical practice to subcategorize PE into mild versus severe based on the absence/presence of severe hypertension, neurological/renal/cardiac impairment, or signs of HELLP. Because complement activation seems to be present in both mild and severe cases, this may represent a common pathway to proximal tubular damage across the PE spectrum. In the current study, this hypothesis is supported by increased urinary excretion of other markers of proximal tubular injury, KIM-1 and A1M, in the PE cases as compared with the GH and NC groups (12). A1M is a glycoprotein produced mainly by the liver. Its function is to bind and degrade free heme and reduce free methemoglobin (7, 8, 32, 34). It also has antioxidant properties, binding downstream reactive oxygen species (ROS), and radicals produced by cell-free hemoglobin (6, 9, 23, 37). A1M under normal circumstances is filtered by the glomerulus and reabsorbed by the proximal tubule (5, 11, 39). Plasma concentrations of A1M were previously observed to increase among patients with PE, perhaps as a response to the increases in cell-free hemoglobin, heme, and ROS that have been observed in this patient population (22) and which can contribute to oxidative stress, systemic endothelial dysfunction, and renal injury. Although A1M does not cause renal damage per se, its reabsorption is decreased in cases of tubular injury or dysfunction, and increased concentrations are found in the urine (39). In contrast, complement C5b-9, also known as the membrane attack complex, is activated in response to renal ischemia and causes direct renal cell injury and necrosis (51). Complement regulatory proteins secreted by the placenta during normal pregnancy successfully inhibit the heightened complement-mediated immune response at various steps in the activation cascade (21, 41). The systemic inflammatory response in PE generated by placental ischemia, however, causes complement dysregulation (14, 33). Complement proteins, including C5b-9, can either be filtered through injured glomeruli or directly activated at the level of the proximal tubule (13, 27, 36). C5b-9 has been shown to directly cause renal cell injury in animal models by inserting into target cell membranes (10, 44, 51). In addition, it activates neutrophils and promotes the release of ROS and cytokines causing further injury (24, 28, 45). Because we did not find elevated concentrations of C5b-9 at the end of the second trimester, a time when podocyturia (a marker of glomerular damage) was demonstrated in these same patients with
PE in a previous study, our results suggest that PE podocyte injury is not temporally associated with terminal complement activation before the manifestation of clinical disease (16).

Contrary to Burwick et al. (12) and Xiao et al. (50), who found elevated concentrations of KIM-1 and RBP in PE compared with normal controls at the time of delivery, we did not observe significant differences in KIM-1 or RBP concentrations between the PE patients and NC. One possible factor contributing to this could be greater disease severity in these previous studies. Indeed, gestational age at delivery in the PE group was earlier in both the study by Burwick et al. (32.7 \pm 4.0 \text{ wk}) (12) and the study by Xiao et al. (36.44 \pm 1.83 \text{ wk}) (50). Interestingly, we have noticed increased concentrations of RBP at both time points (end of 2nd trimester and delivery) in the majority of patients in all groups compared with the normal nonpregnant range. This low-molecular-weight protein is facilitate the early diagnosis of AKI in pregnancy and establish pregnancy-specific normal values that will facilitate the early diagnosis of AKI in pregnancy and characterize a panel of markers of tubular injury among different PE phenotypes (e.g., early vs. late, placental versus maternal, with and without dysregulation of angiogenesis) that may facilitate recognition of subsets of patients for whom specific therapies, such as targeting the complement cascade, may be beneficial. Finally, contrary to evidence of glomerular injury in PE before the development of clinical symptoms, our results indicate that tubular damage that occurs with PE does not predate the clinical disease, as urinary biomarkers of tubular damage were not elevated at the end of the second trimester in the PE patients.

**Perspectives and Significance**

Our findings confirm the hypothesis of the presence of tubular dysfunction in PE. Urinary excretions of A1M and C5b-9 were elevated at the time of delivery in our PE group compared with the GH and NC groups. Elevated excretion of these markers does not appear to antedate the clinical onset of disease and was not accompanied by increased urinary excretion of the tubular cell injury markers KIM1, IGFBP-7, or TIMP-2. These results suggest the possibility of subclinical heme and complement-mediated mechanisms of proximal tubular injury in PE.

**GRANTS**

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


The reference text is a list of scientific papers and articles related to the study of preeclampsia. The papers cover topics such as hypertension in pregnancy, complement activation, and the role of various proteins and compounds in the disease. The references are cited in the text to support the claims and findings made in the study.