Increased urinary levels of podocyte glycoproteins, matrix metalloproteinases, inflammatory cytokines, and kidney injury biomarkers in women with preeclampsia

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Wang Y, Gu Y, Loyd S, Jia X, Groome LJ. Increased urinary levels of podocyte glycoproteins, matrix metalloproteinases, inflammatory cytokines, and kidney injury biomarkers in women with preeclampsia. Am J Physiol Renal Physiol 309:F1009–F1017, 2015. First published October 14, 2015; doi:10.1152/ajprenal.00257.2015.—To investigate kidney injury in preeclampsia, we analyzed 14 biomarkers in urine specimen from 4 groups of pregnant women (normotensive pregnant women and those with pregnancy complicated with chronic hypertension or mild or severe preeclampsia). These biomarkers included 1) podocyte glycoproteins nephrin and podocalyxin, 2) matrix metalloproteinase (MMP)-2 and MMP-9 and their inhibitor tissue inhibitor of metalloproteinase-2, 3) inflammatory molecules and cytokines soluble VCAM-1, TNF-α, soluble TNF receptor receptor-1, IL-6, IL-8, IL-10, and IL-18, and 4) kidney injury biomarkers neutrophil gelatinase-associated lipocalin and kidney injury molecule-1. Postpartum urine specimens (6–8 wk) from normotensive women and those with severe preeclampsia were also evaluated. We found that, first, urine levels of nephrin, MMP-2, MMP-9, and kidney injury molecule-1 were significantly higher before delivery in severe preeclampsia than normotensive groups. The increased levels were all reduced to levels similar to those of the normotensive control group in postpartum specimens from the severe preeclampsia group. Second, soluble VCAM-1, soluble TNF receptor-1, and neutrophil gelatinase-associated lipocalin levels were significantly increased in the severe preeclampsia group compared with the normotensive control group before delivery, but levels of these molecules were significantly reduced in postpartum specimens in both groups. Third, IL-6 and IL-8 levels were not different between preeclampsia and normotensive groups but significantly increased in pregnancy complicated with chronic hypertension. Finally, tissue inhibitor of metalloproteinase-2 and IL-18 levels were not different among the study groups before delivery but were significantly reduced in postpartum specimens from normotensive controls. Our results indicate that the kidney experiences an increased inflammatory response during pregnancy. Most interestingly, tubular epithelial cell injury may also occur in severe preeclampsia. These biomarkers could be used to assess podocyte or tubular injury and kidney inflammatory responses during pregnancy and to evaluate postpartum kidney injury recovery in pregnancy-complicated disorders.

podocyte glycoproteins; kidney inflammatory response; pregnancy; tubular injury; preeclampsia

PREECLAMPSIA is a pregnancy-specific disorder in humans that is characterized by high blood pressure and a large amount of protein in the urine. This disorder occurs after 20 wk of gestation and affects 5–7% of pregnancies worldwide. Preeclampsia increases the risk of poor outcomes for both the mother and baby. Epidemiology studies have also demonstrated that women who had preeclampsia have increased risks of cardiovascular diseases and end-stage kidney diseases later in life (4, 15, 26). It is well known that the kidney is a target organ injured in preeclampsia in association with a characteristic glomerular lesion, endotheliosis, and it is believed that preeclampsia is perhaps the most common glomerular disease in the world (24).

Recently, glomerular podocyte shedding has emerged as a significant kidney lesion in preeclampsia. Several studies have shown that not only podocytes but also podocyte glycoproteins are shed into urine in preeclampsia (2, 7, 27, 30). These findings are consistent with the facts of reduced podocyte glycoprotein nephrin and podoplanin expression in kidney tissues from women who had preeclampsia (6, 29). Moreover, soluble podocyte glycoproteins, such as nephrin and podocalyxin levels, were also significantly higher in urinary specimen from preeclamptic than from normotensive pregnant women (27). Podocyte shedding and/or podocyte protein shedding in preeclampsia indicate that other than glomerular endotheliosis, podocyte injury also contributes to the renal dysfunction in this pregnancy disorder (13). The loss of podocytes has a significant impact in preeclampsia. It weakens and disturbs the ability of the renal barrier to retain plasma protein in the circulation and subsequently results in proteinuria. Although changes in proteinuria may reflect functional as well as morphological alterations in kidneys during preeclampsia, the pathophysiological processes, such as the factors that are responsible for podocyte shedding, inflammatory mediators that may be involved in renal dysfunction, and kidney injury biomarkers that are altered in preeclampsia, are largely unknown.

To study kidney injury in preeclampsia, we analyzed 14 biomarkers, including 1) podocyte glycoproteins nephrin and podocalyxin, 2) matrix metalloproteinase (MMP)-2 and MMP-9 and their inhibitor tissue inhibitor of metalloproteinase (TIMP)-2, 3) inflammatory molecules and cytokines soluble (s)VCAM-1, TNF-α, soluble TNF receptor (sTNFR)-1, IL-6, IL-8, IL-10, and IL-18, and 4) kidney injury biomarkers neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule (KIM)-1, in urine specimens from normotensive pregnant women and pregnant women complicated with chronic hypertension, mild preeclampsia, and severe preeclampsia. We found different patterns of podocyte glycoproteins, inflammatory mediators and cytokines, and kidney injury biomarkers in urine specimens between preeclampsia and pregnancy complicated with chronic hypertension compared with normotensive pregnant controls. Abnormally elevated urine biomarkers in severe preeclampsia were completely or partially reduced 6–8 wk after delivery to levels comparable with those
of normal pregnant subjects. Our results showed that increased renal secretion of MMPs might be responsible for podocyte or podocyte glycoprotein shedding in preeclampsia. Our results also indicate that the kidney experiences an increased inflammatory response during pregnancy, becoming more intense in preeclampsia. Moreover, the increased urine levels of NGAL and KIM-1 in severe preeclampsia suggest that tubular function is also disturbed in preeclampsia and is associated with the severity of the disease.

MATERIALS AND METHODS

Patient information and urine collection. This study was approved by the Institutional Review Board for human research at Louisiana State University Health Sciences Center (Shreveport, LA) and conducted in the Department of Obstetrics and Gynecology. Written informed consent was obtained. A total of 88 pregnant women were recruited in the study, including 28 women with normotensive pregnancy, 15 women with pregnancy complicated with chronic hypertension, 11 women with mild preeclampsia, and 34 women with severe preeclampsia. Spot urine specimens were collected at the time of enrollment at the Women and Children Clinic or at the Labor and Delivery Unit of Louisiana State University Health Sciences Center. Among the study subjects, urine specimens were also obtained from 11 normotensive women and 9 severe preeclamptic pregnant women 6–8 wk after delivery during their routine postpartum visit at the Women and Children Clinic. Freshly obtained urine specimens were centrifuged at 1,500 rpm for 10 min within 30 min of collection, aliquoted, and stored at −70°C until assayed. Diagnoses for normotensive pregnancy, pregnancy complicated with chronic hypertension, mild preeclampsia, or severe preeclampsia followed American College Obstetrics and Gynecology criteria (22). No patient had any signs of infection. Smokers and women with pregnancy complicated by nephritic syndrome, diabetic mellitus, or gestational diabetes were excluded from the study. Patient clinical information was extracted from medical records and is shown in Table 1.

Dipstick detection for urine protein, pH, and gravity. Urine protein, pH, gravity, etc. were detected by Dipstick (Siemens Multistix 10SG) in all urine specimens before being aliquoted for storage at −70°C. Urine protein was positive for all study subjects in mild and severe preeclamptic groups. The range of urine pH was from 5.0 to 8.5, and the range of urine gravity was from 1.000 to 1.030.

Biomarkers evaluated in urine specimens. A total of 14 biomarkers were analyzed in urine specimens, including 1) podocyte glycoproteins nephrin and podocalyxin, 2) MMP-2, MMP-9, and TIMP-2, 3) inflammatory molecules and cytokines sVCAM-1, TNF-α, sTNFR-1, IL-6, IL-8, IL-10, and IL-18, and 4) kidney injury biomarkers NGAL and KIM-1. All biomarkers were measured by ELISA. Assay information for sources of ELISA kits, standard curve range, sample dilution factors, etc. are shown in Table 2. All assays were performed following the manufacturer’s instructions. All specimens were measured in duplicate. Within-assay and between-assay variations were <8% for all assays. Variations in urine pH and gravity had no effect on their ELISAs.

Measurement of urine creatinine concentrations. Urine creatinine concentration was also measured in each sample. The creatinine ELISA kit was purchased from Arbor Assay (Ann Arbor, MI). All samples were measured in duplicate. Creatinine concentration was used to normalize urine levels of detected biomarkers for each sample.

Statistical analysis. Clinical demographic data are presented as means ± SD. Urinary concentrations of podocalyxin, MMP-2, MMP-9, TIMP-2, sVCAM-1, sTNFR-1, IL-18, NGAL, and KIM-1 were adjusted with urinary creatinine concentration and are expressed as means ± SE (in ng/mg creatinine). Data for nephrin are expressed as means ± SE (in μg/mg creatinine). Data for IL-6 and IL-8 are expressed as means ± SE (in pg/mg creatinine). Statistical analysis was performed with ANOVA using Prism 5 computer software (GraphPad Software, La Jolla, CA). A Student-Newman-Keuls test was used as a post hoc test. A Mann-Whitney test was used to compare results between normal and preeclamptic groups. A paired t-test was used to compare biomarker concentrations within each group before delivery and 6–8 wk after delivery. The Pearson product-moment correlation coefficient (Pearson r) was used to analyze the correlation of urine biomarkers with the urine proteinuria-to-creatinine ratio in severe preeclampsia. P values of <0.05 were considered statistically significant.

RESULTS

Patient clinical data. Clinical information, including maternal age, racial status, gravidity, body mass index, blood pressure,
gestational age at urine collection and delivery, and delivery mode, was obtained by chart review. The urine protein-to-creatinine ratio and serum creatinine levels were also obtained by chart review. Clinical characteristics are shown in Table 1. There were no significant differences in maternal age, racial status, and body mass index among the study groups. Maternal systolic and diastolic blood pressures were significantly higher in women in pregnancy complicated with chronic hypertension, mild preeclampsia, and severe preeclampsia groups than in the normotensive group (P < 0.01). The urine protein-to-creatinine ratio was significantly higher in the severe preeclamptic group than in the mild preeclamptic group. There was a higher rate of cesarean section deliveries in the severe preeclampsia group than in the rest of the study groups. Clinical information for normotensive and severe preeclamptic subjects whose urine specimens were studied both before delivery and 6–8 wk after delivery was also reviewed. Blood pressure returned to normotensive levels and positive proteinuria also resolved or diminished to trace levels in severe preeclamptic subjects 6–8 wk after delivery (data not shown). There were three subjects in the normotensive group that experienced preeclampsia in their previous pregnancy but none in the pregnancy complicated with chronic hypertension group.

Increased urine nephrin and podocalyxin levels in severe preeclampsia. Two soluble podocyte glycoproteins, nephrin and podocalyxin, were measured. Nephrin is a podocyte slit adhesion protein, and podocalyxin is a negatively charged glycoprotein on the surface of podocytes. As shown in Table 3, urine concentrations of nephrin and podocalyxin were significantly higher in women with severe preeclampsia than in normotensive pregnant women (P < 0.01). Intriguingly, urine nephrin levels were not different in pregnant women complicated with chronic hypertension or mild preeclampsia compared with normotensive pregnant women. However, urine podocalyxin levels were significantly higher in pregnant women complicated with chronic hypertension and mild preeclampsia than in normotensive pregnant women (P < 0.01). The discrepancy between nephrin and podocalyxin shedding in pregnancy complicated with chronic hypertension and preeclampsia provides evidence of different pathophysiological processes that occur in the kidney between the two pregnancy-complicated disorders.

Increased urine MMP-2 and MMP-9 but not TIMP-2 levels in severe preeclampsia. To determine if altered protease function may contribute to podocyte glycoprotein shedding, two major MMPs, MMP-2 and MMP-9, were measured. Similar to nephrin, we found that urine MMP-2 and MMP-9 concentrations were significantly higher in women with severe preeclampsia than in normal pregnant women (P < 0.05 or P < 0.01, respectively; Table 3). MMP-2 and MMP-9 levels were slightly, but not significantly, increased in pregnant women complicated with chronic hypertension and mild preeclampsia compared with normal pregnant women. Urine TIMP-2 levels were not different among the groups.

Altered urine inflammatory biomarkers in women with preeclampsia and chronic hypertension. A total of seven molecules and ILs that are associated with increased inflammatory responses were evaluated in urine specimen, including sVCAM-1, TNF-α, its soluble receptor sTNFR-1, and four ILs (IL-6, IL-8, IL-10, and IL-18). The results are shown in Table 3. sVCAM-1 levels were only increased in pregnant women with severe preeclampsia compared with normal pregnant women (P < 0.05). sTNFR-1 levels were increased both in women with severe preeclampsia and in pregnant women complicated with chronic hypertension compared with normal pregnant women (P < 0.05). Although urine IL-6 and IL-8 levels were higher in pregnant women complicated with mild and severe preeclampsia than in normal pregnant women, their levels were only significantly increased in pregnant women complicated with chronic hypertension (P < 0.05). Urine IL-18 levels were not different among the groups. In addition, TNF-α was not detectable in urine specimens. IL-10 was only detected in a few samples (not shown). The different patterns of inflammatory molecules/cytokines in urine specimens among the study groups may represent diverse inflammatory responses and different roles of these molecules in these pregnancy-complicated disorders.
Increased urine NGAL and KIM-1 levels in severe preeclampsia. NGAL, known as lipocalin-2, is an iron-transporting protein that is almost entirely reabsorbed by tubules in the normal kidney (25). KIM-1 is a transmembrane protein that is expressed on the luminal surface of proximal tubules during injury. Our results showed that urine NGAL and KIM-1 levels were significantly higher in women with severe preeclampsia compared to normotensive pregnant women and those with mild or chronic hypertension. Although TIMP-2 levels were not different between normal pregnant women and those with severe preeclampsia (P < 0.01; Table 3). Since both NGAL and KIM-1 have been considered biomarkers for kidney injury (1, 25), the increased urine levels of NGAL and KIM-1 in severe preeclampsia suggest that altered tubular function also occurs in preeclampsia, which is associated with the severity of this pregnancy disorder.

Urine biomarker levels in normotensive pregnant women and women with severe preeclampsia 6–8 wk after delivery. As shown in Table 3, urine levels of podocyte glycoproteins nephrin and podocalyxin, MMP-2 and MMP-9, inflammatory markers sVCAM-1 and sTNFR-1, and kidney injury markers NGAL and KIM-1 were all significantly higher in women with severe preeclampsia than in normotensive pregnant women or than in women complicated with chronic hypertension or mild preeclampsia. Among the study subjects, urine specimens were also collected from 20 women 6–8 wk after delivery (11 normotensive women and 9 women with severe preeclampsic pregnancies). We then measured these biomarkers in postpartum delivery specimens to determine if the abnormal increased urine levels of these biomarkers stayed persistently high or returned to levels comparable with normal pregnant women postpartum. The results are shown in Fig. 1. Figure 1A shows concentrations of nephrin, podocalyxin, MMP-2, MMP-9, and TIMP-2 in normal pregnant women and those with severe preeclampsia before and 6–8 wk after delivery. We found that urine levels of nephrin, podocalyxin, MMP-2, and MMP-9 were not different in normal pregnant women before and after delivery. However, the increased urine levels of nephrin, podocalyxin, MMP-2, and MMP-9 in severe preeclampsia before delivery were all significantly reduced 6–8 wk after delivery to levels similar to those in normotensive pregnant women. Our result showed that urine podocalyxin levels were normalized postpartum in preeclampsia, which is consistent with previously published work (19). These results suggest that kidney barrier function is recovered or is in the process of recovering during the postpartum period. Kidney function recovery is consistent with maternal systemic blood pressure returning to normal levels in patients with preeclampsia. Although TIMP-2 levels were not different between normal and severe preeclampsia before delivery, TIMP-2 levels were significantly reduced in both normal and severe preeclamptic pregnant women 6–8 wk after delivery. Because TIMP-2 is a native inhibitor for both MMP-2 and MMP-9 (14), the reduced TIMP-2 levels after delivery suggest that increased renal se-

<table>
<thead>
<tr>
<th>Markers</th>
<th>Normal Pregnant Women</th>
<th>Pregnant Women Complicated With Chronic Hypertension</th>
<th>Pregnant Women Complicated With Mild Preeclampsia</th>
<th>Pregnant Women Complicated With Severe Preeclampsia</th>
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<tr>
<td>Number of women/group</td>
<td>28</td>
<td>15</td>
<td>11</td>
<td>34</td>
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<td>Nephrin, µg/mg creatinine</td>
<td>0.98 ± 0.20</td>
<td>0.77 ± 0.29</td>
<td>0.52 ± 0.16</td>
<td>4.35 ± 1.09&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Range</td>
<td>0.01-3.20</td>
<td>0.01-3.31</td>
<td>0.02-1.16</td>
<td>0.10–33.85</td>
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<td>Podocalyxin, ng/mg creatinine</td>
<td>12.10 ± 2.72</td>
<td>97.33 ± 21.71&lt;sup&gt;h&lt;/sup&gt;</td>
<td>87.16 ± 23.54&lt;sup&gt;h&lt;/sup&gt;</td>
<td>230 ± 50.96&lt;sup&gt;ce&lt;/sup&gt;</td>
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<td>Range</td>
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<td>24.05-220.60</td>
<td>14.54–943.80</td>
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<td>Matrix metalloproteinase-2, ng/mg creatinine</td>
<td>1.41 ± 0.22</td>
<td>1.80 ± 0.49</td>
<td>2.17 ± 0.25</td>
<td>3.03 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.17-5.32</td>
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<td>0.73-3.44</td>
<td>0.21–11.66</td>
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<td>Matrix metalloproteinase-9, ng/mg creatinine</td>
<td>0.09 ± 0.03</td>
<td>0.29 ± 0.10</td>
<td>0.59 ± 0.26</td>
<td>1.40 ± 0.39&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0-1.18</td>
<td>0.2-4.64</td>
<td>0.01-10.22</td>
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<tr>
<td>Tissue inhibitor of metalloproteinase-2, ng/mg creatinine</td>
<td>2.10 ± 0.33</td>
<td>1.45 ± 0.37</td>
<td>2.41 ± 0.37</td>
<td>2.74 ± 0.70</td>
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<tr>
<td>Range</td>
<td>0.01-6.40</td>
<td>0-3.83</td>
<td>0.73–3.87</td>
<td>0-21.65</td>
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<tr>
<td>Soluble VCAM-1, ng/mg creatinine</td>
<td>11.79 ± 2.36</td>
<td>15.88 ± 3.57</td>
<td>16.24 ± 5.53</td>
<td>28.95 ± 5.28&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Range</td>
<td>0.19-38.46</td>
<td>0.51-36.10</td>
<td>0.35-64.59</td>
<td>2.10–145.13</td>
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<td>Soluble TNF receptor-1, ng/mg creatinine</td>
<td>2.27 ± 0.37</td>
<td>5.66 ± 1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.32 ± 0.67</td>
<td>3.99 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.24-29.44</td>
<td>0.97-6.93</td>
<td>0.71-8.74</td>
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<tr>
<td>IL-8, pg/mg creatinine</td>
<td>1.01 ± 0.32</td>
<td>4.51 ± 1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.49 ± 1.85</td>
<td>2.60 ± 0.67</td>
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<td>Range</td>
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<td>0.22-18.8</td>
<td>0-16.03</td>
<td>0-15.54</td>
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<td>IL-8, pg/mg creatinine</td>
<td>39.17 ± 9.19</td>
<td>177.70 ± 77.65&lt;sup&gt;h&lt;/sup&gt;</td>
<td>131.10 ± 36.39</td>
<td>99.26 ± 25.65</td>
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<td>Range</td>
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<td>0-877.20</td>
<td>4.21-434.70</td>
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<td>IL-18, ng/mg creatinine</td>
<td>80.35 ± 8.37</td>
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<td>75.39 ± 15.23</td>
<td>68.30 ± 11.01</td>
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<td>Kidney injury molecule-1, ng/mg creatinine</td>
<td>0.41 ± 0.07</td>
<td>0.67 ± 0.16</td>
<td>0.72 ± 0.11</td>
<td>1.42 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Range</td>
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<td>0.07-1.90</td>
<td>0.33-1.59</td>
<td>0.08-9.14</td>
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<td>Neutrophil gelatinase-associated lipocalin, ng/mg creatinine</td>
<td>21.32 ± 3.80</td>
<td>37.47 ± 6.54</td>
<td>31.50 ± 9.44</td>
<td>64.21 ± 15.61&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>Range</td>
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<td>6.68-78.68</td>
<td>3.19-93.98</td>
<td>8.85-352</td>
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<tr>
<td>Urine creatinine, mg/ml</td>
<td>0.99 ± 0.10</td>
<td>1.23 ± 0.20</td>
<td>0.66 ± 0.08</td>
<td>0.82 ± 0.08</td>
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<tr>
<td>Range</td>
<td>0.21-2.42</td>
<td>0.18-2.91</td>
<td>0.41-1.28</td>
<td>0.17-2.08</td>
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</tbody>
</table>

Values are as means ± SE. *P < 0.05 and #P < 0.01, other groups vs. the normal pregnancy group; ①P < 0.05 and ②P < 0.01, severe preeclampsia vs. mild preeclampsia groups; ③P < 0.05, severe preeclampsia vs. chronic hypertension.
cretion of TIMP-2 during pregnancy could be a compensative mechanism to counteract protease insult to podocytes or glomerular/tubular cells.

Figure 1B shows urine concentrations of sVCAM-1, sTNFR-1, IL-18, NGAL, and KIM-1 in normal pregnancy and severe preeclampsia before and 6–8 wk after delivery. Urine levels of both sVCAM-1 and sTNFR-1 were significantly reduced 6–8 wk postpartum in normal and severe preeclampsia groups ($P < 0.01$). Urine IL-18 levels were not different before and 6–8 wk after delivery in women with severe preeclampsia and normal pregnancy ($P > 0.05$). Urine NGAL levels were not different before and 6–8 wk after delivery in women with severe preeclampsia and normal pregnancy ($P > 0.05$). Urine KIM-1 levels were significantly reduced at 6–8 wk after delivery in women with severe preeclampsia ($P < 0.05$) but not in normal pregnancy ($P > 0.05$). There were no differences in KIM-1 levels in normal pregnant women before and 6–8 wk after delivery, but KIM-1 levels were significantly reduced at 6–8 wk after delivery in women with severe preeclampsia ($P < 0.05$) compared to normal pregnancy ($P > 0.05$).
MMP-2 and MMP-9 levels were increased in severe pre-eclampsia, and severe preeclampsia compared with normotensive pregnancies (P < 0.01). The pattern for KIM-1 was similar to nephrin. There were no differences in KIM-1 levels before and 6–8 wk after delivery in normal pregnant women, but the increased urine KIM-1 levels in severe preeclampsia before delivery were significantly reduced 6–8 wk postpartum (P < 0.05). It is known that NGAL and KIM-1 are tubular injury biomarkers. Therefore, our results suggest that tubular injury occurs in women with severe preeclampsia.

**Correlation of urine biomarkers and the urine protein-to-creatinine ratio in severe preeclampsia.** We also determined if there was a correlation between urinary glomerular and tubular markers with the urinary albumin-to-creatinine ratio in patients with severe preeclampsia. The results are shown in Fig. 2. Figure 2A shows the correlation of urine levels of nephrin, podocalyxin, MMP-2, MMP-9, and TIMP-2 with the urine albumin-to-creatinine ratio. Figure 2B shows the correlation of urine levels of sVCAM, sTNFR-1, IL-18, NGAL, and KIM-1 with the urine albumin-to-creatinine ratio. Interestingly, podocyte glycoprotein nephrin and podocalyxin and tubular biomarkers NGAL and KIM-1 were positively correlated with the urinary albumin-to-creatinine ratio in patients with severe preeclampsia. For inflammatory markers, only sVCAM levels were correlated with the urinary albumin-to-creatinine ratio, whereas sTNFR1 and IL-18 were not. In addition, TIMP-2, but not MMP-2 and MMP-9, was correlated with the increased urinary albumin-to-creatinine ratio in patients with severe preeclampsia. These results suggest that podocyte and tubular cell injury are associated with glomerular barrier dysfunction in preeclampsia. However, increased inflammatory responses and increased MMP secretion may not.

**DISCUSSION**

Glomerular endotheliosis and podocyte shedding play a central role in kidney barrier dysfunction in preeclampsia. Urine podocyte proteins of nephrin and podocalyxin have been considered biomarkers for preeclampsia (21). To further evaluate kidney injury in preeclampsia, in the present study, we measured inflammatory mediators, MMPs, and kidney injury biomarkers in urine specimens from normotensive pregnant women and pregnant women complicated with chronic hypertension, mild preeclampsia, or severe preeclampsia. We sought to determine if increased podocyte glycoprotein shedding is associated with increased MMP secretion in preeclampsia, whether the inflammatory response is increased in the kidney in preeclampsia, and whether tubular injury occurs in preeclampsia. Several significant findings were obtained.

First, we found different patterns of urine nephrin and podocalyxin levels in preeclampsia and pregnancy complicated with chronic hypertension. Urine nephrin levels were significantly increased in severe preeclampsia, whereas urine podocalyxin levels were significantly increased in women with pregnancy complicated with chronic hypertension, mild preeclampsia, and severe preeclampsia compared with normotensive pregnant women. Interestingly, similar patterns of urine MMP-2 and MMP-9 to nephrin were noticed, in which urine MMP-2 and MMP-9 levels were increased in severe preeclampsia but not in pregnancy complicated with chronic hypertension or mild preeclampsia. MMP-2 and MMP-9 belong to the collagenase type IV family, and both degrade collagen type IV, the major structural component of basement membranes. Nephrin is a transmembrane protein of podocyte slit diaphragms. Loss of nephrin results in podocyte shedding and proteinuria. Because TIMP-2 is an innate inhibitor for both MMP-2 and MMP-9 (14), we also determined urine TIMP-2 levels. Although urine TIMP-2 levels were not different among the study groups before delivery, their levels were significantly reduced in both normotensive and severe preeclamptic pregnancies 6–8 wk after delivery, which suggest that the increased renal secretion of TIMP-2 could be a compensative mechanism to protect glomerular/kidney tissue or the basement membrane from metallopeptidase insult during pregnancy. In the present study, we did not specifically investigate the mechanism of renal MMP-2- and MMP-9-mediated podocyte nephrin shedding in preeclampsia. However, our results of reduced urine nephrin and podocalyxin and MMP-2 and MMP-9 levels 6–8 wk after delivery in severe preeclampsia indicate that increased renal secretion of MMP-2 and MMP-9 could play, at least in part, a role in podocyte shedding or podocyte glycoprotein shedding in preeclampsia (27, 30).

Podocalyxin is a major negatively charged glycoprotein on the apical cell membrane of podocytes. Podocalyxin shedding into urine is believed to be an indication of podocyte injury (10). Urine podocalyxin levels were increased not only in patients with preeclampsia but also in pregnant women complicated with chronic hypertension. The differences in podocalyxin and nephrin shedding between pregnancy complicated with chronic hypertension and severe preeclampsia found in our study subjects imply that increased podocalyxin shedding could be a more sensitive marker of podocyte injury in kidney-related diseases in general. In fact, increased urinary levels of podocalyxin have been reported in several renal-related diseases, such as in patients with diabetic nephropathy (9) and IgA nephropathy (3). However, nephrin shedding may specifically represent podocyte slit diaphragm damage in severe preeclampsia.

The second important finding is the different phenotype of increased kidney inflammatory responses during pregnancy. This notion is supported by the following results: 1) increased urine levels of sVCAM-1 in severe preeclampsia, 2) increased urine levels of IL-6 and IL-8 in pregnancy complicated with chronic hypertension, and 3) increased urine levels of sTNFR-1 in both severe preeclampsia and pregnancy complicated with chronic hypertension. More importantly, urine levels of sVCAM-1 and sTNFR-1 were reduced not only in pregnant women with severe preeclampsia but also in normotensive pregnant women 6–8 wk after delivery (Fig. 2B). These results suggest that an increased kidney inflammatory response not only occurs in preeclampsia but also in normal pregnancy as well. This notion is also supported by the reduction of urine IL-18 levels 6–8 wk postpartum in normal pregnant women. VCAM-1 is expressed by the renal vessel endothelium and could be enzymatically cleaved or excreted into urine during the process of an increased glomerular inflammatory response. In addition, maternal sVCAM-1 could also be filtered through a leaky glomerulus and contributes to increased urine levels in preeclampsia. TNFR-1 is one of the major receptors for TNF-α and is expressed on infiltrating leukocytes and kidney cells.
during kidney inflammation. sTNFR-1 is more stable and easier to detect in urine than TNF-α and thus can serve as a surrogate marker of TNF-α activity in kidney diseases (25). Studies have shown that serum or urine levels of sTNFR-1 were increased in acute and chronic kidney inflammatory diseases and diabetic nephropathy (17, 28). Therefore, the increased urine sVCAM-1 levels might represent, at least in part, glomerular endothelial dysfunction in severe preeclamp-
nia, whereas increased urine levels of sTNFR-1 might reflect a chronic kidney inflammatory response in pregnancy complicated with chronic hypertension.

Unfortunately, we did not have enough postpartum urine specimens from pregnant women complicated with chronic hypertension. Thus, it is not known whether the elevated urine levels of IL-6 and IL-8 before delivery would be reduced to normotensive control levels or persist at high levels during the postpartum period in pregnant women complicated with chronic hypertension. Further investigation is warranted. IL-18 is a proinflammatory cytokine that is produced by kidney cells (1). Increased urine IL-18 levels are considered to be a specific marker for acute tubular necrosis in the postischemic kidney (20). In our study, urine IL-18 levels were not different among the study groups before delivery, but levels were reduced 6–8 wk later in normotensive control patients. Although this phenomenon may support the idea that the kidney experiences an increased inflammatory response in normal pregnancy, urine IL-18 levels may not be a viable biomarker for preeclampsia. Nonetheless, the different patterns of increased sVCAM-1 and sTNFR-1 in severe preeclampsia and increased IL-6 and IL-8 in pregnancy complicated with chronic hypertension provide considerable evidence of phenotypic differences of an increased kidney inflammatory response in pregnancy-complicated disorders.

Another significant finding of our study is elevated urine levels of NGAL and KIM-1 in severe preeclampsia compared with those in normal pregnant women and in pregnant women complicated with chronic hypertension and mild preeclampsia. Our results are different from what has been previously reported by Odum et al. (18). They found that urine secretion of NGAL was higher in normal pregnant women than in women with preeclamptic pregnancies (18). The reason for the discrepancy between our results and theirs is not known. We used a standard ELISA assay and they used an ARCHITECT NGAL assay. However, our findings of elevated urine levels of NGAL are consistent with increased urine levels of KIM-1 as well as podocyte proteins nephrin and podocalyxin in severe preeclampsia. In addition, sex- and age-related differences in urine NGAL levels have been noticed, with women or older people (>60 yr old) tentatively having higher NGAL concentrations (5). Both NGAL and KIM-1 are considered biomarkers for acute and chronic kidney injury. NGAL is an iron-transporting protein that is synthesized and secreted by tubular epithelial cells of proximal and distal segments. NGAL is almost entirely reabsorbed by tubules in the normal kidney. In the setting of acute tubular injury, NGAL levels increase rapidly and can be detected in both urine and plasma (25). KIM-1 is a putative epithelial cell adhesion molecule containing an Ig domain (12). The KIM-1 level is very low in the normal kidney but has been found to be dramatically increased in the postischemic kidney (8). KIM-1 has been identified as the first nonmyeloid phosphatidyserine receptor that confers a phagocyte phenotype on injured epithelial cells both in vivo and in vitro experiments (11). Like NGAL, the urinary KIM-1 level is believed to be an early indicator of acute kidney injury (23). In fact, both NGAL and KIM-1 have been considered as emerging biomarkers for assessing toxic nephropathy and acute kidney injury in newborn babies (16). In the present study, we found that urine levels of both NGAL and KIM-1 were not different between chronic hypertension or mild preeclampsia and normotensive pregnant control groups before delivery but were significantly increased in the severe preeclampsia group. Interestingly, urine levels of NGAL were reduced in both normotensive and severe preeclamptic pregnancies 6–8 wk after delivery. In comparison, no changes in urine KIM-1 levels were observed before and 6–8 wk after delivery in normotensive pregnant women, but the increased urine KIM-1 levels before delivery in severe preeclampsia were significantly reduced 6–8 wk after delivery. The increased urine levels of NGAL and KIM-1 in severe preeclampsia provide valuable evidence that not only glomerular cells (endothelial cells and podocytes) but also the function of tubule epithelial cells are disturbed in severe preeclampsia. The increased urine KIM-1 levels may also indicate ischemic kidney injury in severe preeclampsia, suggesting that urine KIM-1 could be a relatively sensitive biomarker of kidney injury associated with severity in preeclampsia.

Our data also showed that urine levels of podocyte glycoproteins nephrin and podocalyxin and tubular injury markers NGAL and KIM-1 were correlated with the urine protein-to-creatinine ratio in severe preeclampsia. The urine protein-to-creatinine ratio is commonly used to determine if excess levels of protein are present in urine or to estimate the urine protein excretion rate. The positive correlation of these urine biomarkers with the protein-to-creatinine ratio in severe preeclampsia provides further evidence that increased podocyte glycoprotein shedding and tubular injury marker release are significantly associated with glomerular barrier/kidney dysfunction in severe preeclampsia. Urine levels of sVCAM, but not sTNFR-1 and IL-18, were also correlated with the urine protein-to-creatinine ratio. This suggests that increased kidney cytokine secretion may not be directly associated with glomerular barrier dysfunction in preeclampsia but that glomerular endothelium dysfunction is, since the glomerular endothelium is part of the glomerular barrier structure and glomerular endotheliosis is a significant pathophysiological event in preeclampsia. Whether increased urine cytokine secretion, such as sTNFR-1, reflects an overall increased systemic inflammatory response during pregnancy and/or an exaggerated inflammatory response in preeclampsia needs further investigation. It is also noted that although urine TIMP-2 levels were not different among the study groups during pregnancy, TIMP-2 levels were significantly reduced postpartum in both normotensive and severe preeclamptic pregnancies. Moreover, TIMP-2 levels were also significantly correlated with the protein-to-creatinine ratio in severe preeclampsia, whereas MMP-2 and MMP-9 were not, even though they were significantly increased in severe preeclampsia. The reason for this is not clear but could reflect a phenomenon of a compensative reaction of TIMP-2 to protect kidney injury in preeclampsia.

In summary, the results obtained from this study reveal that kidneys undergo an increased inflammatory response during pregnancy. The different patterns of urine levels of inflammatory molecules and cytokines between pregnancies complicated with chronic hypertension and preeclampsia support the idea of phenotypic differences of an increased kidney inflammatory response in pregnancy-complicated disorders. The consistency of increased urine levels of MMP-2 and MMP-9 with increased urine nephrin levels provide evidence that altered metallopeptidase activities may play, at least in part, a role in podocyte slit protein nephrin shedding in severe preeclampsia. Most notably, the increased urine levels of NGAL and KIM-1
in severe preeclampsia suggest that other than glomerular endothelial cells and podocytes, the function of tubular epithelial cells may also be disturbed, which contributes to kidney injury in severe preeclampsia. Although we did not include nonpregnant patients in the present study, our results of reduced urine inflammatory, glomerular, and tubular injury biomarker levels postpartum in both normotensive and severe preeclamptic subjects clearly showed the significance of these biomarkers between the nonpregnant state (postpartum) and pregnant conditions. Therefore, we believe that urine levels of these biomarkers could be used to evaluate the degree of increased inflammatory responses, function of glomerular podocytes/endothelial cells, and even tubular epithelial cells during pregnancy and pregnancy-complicated disorders. Further studies with a large sample size are warranted to establish a convenient test to monitor kidney function before and after delivery, which would provide valuable information for clinical interventions to reduce kidney or cardiovascular diseases in women later in life, such as preeclampsia, gestational diabetes, and pregnancy complicated with chronic hypertension, as well as other chronic kidney diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.W., Y.G., and S.L. conceived and designed the research; Y.W., Y.G., S.L., and X.J. performed experiments; Y.W., Y.G., S.L., and X.J. analyzed data; Y.W., Y.G., S.L., and X.J. interpreted results of research; Y.W., Y.G., X.J., and L.J.G. approved final version of manuscript.

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