Preeclampsia

Complement Activation and Kidney Injury Molecule-1–Associated Proximal Tubule Injury in Severe Preeclampsia

Richard M. Burwick, Sarah Rae Easter, Hassan Y. Dawood, Hidemi S. Yamamoto, Raina N. Fichorova, Bruce B. Feinberg

Abstract—Kidney injury with proteinuria is a characteristic feature of preeclampsia, yet the nature of injury in specific regions of the nephron is incompletely understood. Our study aimed to use existing urinary biomarkers to describe the pattern of kidney injury and proteinuria in pregnancies affected by severe preeclampsia. We performed a case–control study of pregnant women from Brigham and Women’s Hospital from 2012 to 2013. We matched cases of severe preeclampsia (n=25) 1:1 by parity and gestational age to 2 control groups with and without chronic hypertension. Urinary levels of kidney injury molecule-1 and complement components (C3a, C5a, and C5b-9) were measured by enzyme-linked immunosorbent assay, and other markers (albumin, β2 microglobulin, cystatin C, epithelial growth factor, neutrophil gelatinase–associated lipocalin, osteopontin, and uromodulin) were measured simultaneously with a multiplex electrochemiluminescence assay. Median values between groups were compared with the Wilcoxon signed-rank test and correlations with Spearman correlation coefficient. Analysis of urinary markers revealed higher excretion of albumin and kidney injury molecule-1 and lower excretion of neutrophil gelatinase–associated lipocalin and epithelial growth factor in severe preeclampsia compared with chronic hypertension and healthy controls. Among subjects with severe preeclampsia, urinary excretion of complement activation products correlated most closely with kidney injury molecule-1, a specific marker of proximal tubule injury (C5a: r=0.60; P=0.001; and C5b-9: r=0.75; P<0.0001). Taken together, we describe a pattern of kidney injury in severe preeclampsia that is characterized by glomerular impairment and complement-mediated inflammation and injury, possibly localized to the proximal tubule in association with kidney injury molecule-1. (Hypertension. 2014;64:833-838.) ● Online Data Supplement

Key Words: complement membrane attack complex ■ complement system proteins ■ preeclampsia ■ pregnancy

Kidney injury with proteinuria is a characteristic feature of preeclampsia, yet the nature of injury in specific regions of the nephron is incompletely understood. Glomerular barrier dysfunction, characterized by glomerular endotheliosis and podocyte loss, has been emphasized in severe preeclampsia because marked albuminuria is a hallmark feature of disease. However, inflammation and injury to the proximal tubule also contributes to disease pathogenesis in many kidney disorders. Considering that the tubulointerstitium accounts for >90% of kidney volume and that tubulointerstitial disease may occur independent of glomerular disease, it is critical that urinary protein assessment in preeclampsia consider both glomerular and nonglomerular patterns of injury.

Although the cause of preeclampsia is incompletely understood, active disease is most often characterized as a multiorgan, systemic inflammatory disorder with endothelial cell activation, intravascular volume depletion, hypertension, and kidney injury. Complement activation is central to the systemic inflammatory response in preeclampsia, possibly in response to apoptotic or necrotic trophoblast debris at the placental interface and in systemic circulation. Marked urinary excretion of complement components in severe preeclampsia suggests that complement-mediated kidney injury is a key feature of disease. To map out potential sites of complement-mediated kidney injury in preeclampsia, we sought to measure a panel of urinary biomarkers linked to specific regions of the nephron among pregnant women with and without preeclampsia.

Methods

We enrolled 25 cases with severe preeclampsia, 25 controls with chronic hypertension, and 25 healthy controls without hypertension from a cohort of women receiving care at Brigham and Women’s Hospital from March 2012 through March 2013. Institutional review board approval was obtained through the Partners Human Research Committee, and subjects gave informed consent. All procedures followed were in accordance with institutional guidelines. Methods

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Preeclampsia cases were recruited from the labor and delivery ward with multiple gestation or major fetal anomalies were excluded. Pregnancies have been described previously,20 but key aspects are summarized below with modifications pertinent to this investigation. Pregnancies with multiple gestation or major fetal anomalies were excluded. Preeclampsia cases were recruited from the labor and delivery ward after 1:2 dilution (C3a, C5a, C5b-9) or no dilution (KIM-1) followed by 1:2 dilution if protein levels exceeded the top standard. Samples were analyzed in duplicate after 1:100 dilution followed by 1:1000 dilution if protein levels exceeded the top standard. Two quality control pools were tested at different dilutions matching the linearity range on each assay plate to assess interassay variation. Intra-assay coefficient of variation was 3.5% (albumin), 6.0% (B2M), 7.6% (cystatin C), 0.79% (EGF), 3.3% (NGAL), 7.3% (osteopontin), and 0.92% (uromodulin); interassay coefficient of variation was 3.2% (albumin), 9.4% (B2M), 7.7% (cystatin C), 4.6% (EGF), 7.5% (NGAL), 2.9% (osteopontin), and 5.7% (uromodulin). The lower limit of detection was a calculated concentration based on a signal 2.5 SDs above the background (zero calibrator blank) and were 226 (albumin), 1.82 (B2M), 9.81 (cystatin C), 0.057 (EGF), 2.66 (NGAL), 69.9 (osteopontin), and 52.5 pg/mL (uromodulin).

Figure 1. Proposed source of urinary biomarkers within the nephron: albumin, β2 microglobulin (B2M), cystatin C, epithelial growth factor (EGF), kidney injury molecule-1 (KIM-1), neutrophil gelatinase–associated lipocalin (NGAL), osteopontin (OPN), and uromodulin (UMOD).

Results

The baseline characteristics of the 3 study groups have been published previously20 and are presented in the Table. Controls were matched to severe preeclampsia cases by parity and gestational age at enrollment.

Absolute urine protein levels of all measured kidney markers in cases and controls are displayed in Figure 2, with concentrations varied for display purposes. Levels of urine albumin, a marker of glomerular barrier dysfunction,2,12 were markedly elevated in subjects with severe preeclampsia compared with both control groups. Albumin levels were no different between controls with chronic hypertension and healthy controls without hypertension. These findings were unchanged after adjustment for urine creatinine (Table S1 in the online-only Data Supplement).

Urinary levels of KIM-1, a specific marker of proximal tubule injury,20 were also significantly greater in severe preeclampsia compared with both control groups. Concentrations of NGAL and EGF, proposed markers of acute kidney injury and tubular damage,28,29,31,32 were significantly lower in severe preeclampsia compared with controls. Levels of cystatin C, a sensitive marker of glomerular filtration because of its small size,26,27 were increased in chronic hypertension but not severe preeclampsia. Other measured urinary markers of kidney injury (B2M, osteopontin, and uromodulin) were not different between any of the study groups. These findings remained unchanged after adjustment for urine creatinine (Table S1).

In this same cohort of subjects, we recently reported that urinary excretion of complement components was increased in severe preeclampsia.20 Thus, we investigated correlations between these previously measured complement markers (C3a, C5a, and C5b-9) and the kidney markers measured in
this analysis. To explore associations in active disease, we focused our analysis on subjects with severe preeclampsia (n=25), and the correlation results are provided in Table S2. Of the kidney markers assessed, only KIM-1 levels correlated significantly with proteinuria, the cause of proximal tubule injury.30,31 The proximal tubule injury in preeclampsia is unclear. In our investigation, urinary albumin excretion was increased in severe preeclampsia compared with healthy controls and 40× greater than controls with chronic hypertension.

In preeclampsia, the integrity of the glomerular basement membrane may be impaired by increased serum levels of soluble fms-like tyrosine kinase-1, which sequesters vascular endothelial growth factor; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; OPN, osteopontin; and UMOD, uromodulin. "Albumin (μg/mL×10); B2M and OPN (ng/mL); cystatin C, EGF, and NGAL (ng/mL×10); KIM-1 (ng/dL), and UMOD (ng/mL×10⁻³); concentrations varied for display purposes. P<0.0001, PE vs healthy controls or CHTN; P=0.02, CHTN vs healthy controls or PE; ❄=0.001, PE vs healthy controls or CHTN; □=0.002, PE vs healthy controls and +=0.01 vs CHTN; ■=0.004, PE vs healthy controls and P=0.003 vs CHTN. White bars indicate healthy controls; gray bars, CHTN; black bars, severe PE.

![Figure 2. Relative concentration of urinary biomarkers in preeclampsia (PE) cases and controls. Median (horizontal line), interquartile range (box), range (whiskers), outliers >1.5 SD from upper quartile excluded for display purposes. B2M indicates β2 microglobulin; CHTN, chronic hypertension; EGF, epithelial growth factor; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; OPN, osteopontin; and UMOD, uromodulin. Albumin (μg/mL×10); B2M and OPN (ng/mL); cystatin C, EGF, and NGAL (ng/mL×10); KIM-1 (ng/dL), and UMOD (ng/mL×10⁻³); concentrations varied for display purposes. P<0.0001, PE vs healthy controls or CHTN; P=0.02, CHTN vs healthy controls or PE; P=0.003 vs CHTN. White bars indicate healthy controls; gray bars, CHTN; black bars, severe PE.

### Discussion

Kidney injury in preeclampsia is most commonly defined by the total excretion of urinary protein in 24 hours or by the ratio of total urine protein to creatinine in a random urine collection.36,38 However, the validity of standard urinary protein measurements for the diagnosis of preeclampsia has been questioned,38,39 partly because the extent of proteinuria in preeclampsia does not correlate well with disease severity. Although the importance of absolute urine protein levels in severe preeclampsia is questioned, our study confirms that there is significant glomerular barrier impairment as indicated by marked albuminuria in active disease. Urinary albumin levels were 75× greater in severe preeclampsia compared with healthy controls and 40× greater than controls with chronic hypertension.

Although the proximal tubule serves a critical role in disease states defined by proteinuria, the cause of proximal tubule injury in preeclampsia is unclear. In our investigation, urinary excretion of B2M and cystatin C was no different, or lower, in subjects with severe preeclampsia. This finding argues against diffuse impairment of proximal tubule reabsorption, which would lead to increased excretion of filtered proteins. Interestingly, we did find that urinary excretion of KIM-1, a specific marker of proximal tubule injury,30 was increased in preeclampsia. KIM-1 is intriguing because it is upregulated by proximal tubule epithelial cells in response to renal ischemia and kidney injury. Expression of KIM-1 converts proximal tubule epithelial cells into professional phagocytes that

### Table. Characteristics of Study Subjects by Group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy Controls (n=25)</th>
<th>CHTN (n=25)</th>
<th>Severe Preeclampsia (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at enrollment,* wk</td>
<td>30.9±4.8</td>
<td>31.3±4.7</td>
<td>32.3±4.2</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>39.5±1.2</td>
<td>38.2±1.6</td>
<td>32.7±4.0</td>
</tr>
<tr>
<td>Peak SBP at enrollment, mm Hg</td>
<td>111±9.5</td>
<td>132±12.1†</td>
<td>176±20.7†</td>
</tr>
<tr>
<td>Peak DBP at enrollment, mm Hg</td>
<td>67.0±7.9</td>
<td>82.3±10.6†</td>
<td>104±11.0†</td>
</tr>
<tr>
<td>Urine total protein/creatinine, mg/mL</td>
<td>0.64 (0–1.3)</td>
<td>0.64 (0.3–1.2)</td>
<td>1.9 (1.6–4.0)</td>
</tr>
<tr>
<td>Nulliparous,* %</td>
<td>68.0</td>
<td>68.0</td>
<td>68.0</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>29.0±5.5</td>
<td>33.6±5.9¶</td>
<td>30.6±5.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6±4.1</td>
<td>34.4±9.2#</td>
<td>31.8±6.0**</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>44.0</td>
<td>52.0</td>
<td>60.0</td>
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<tr>
<td>Black</td>
<td>24.0</td>
<td>32.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>28.0</td>
<td>16.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Other</td>
<td>4.0</td>
<td>0.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Data are mean±SD, comparisons between groups assessed by paired t tests and differences nonsignificant (P>0.05) unless otherwise specified. CHTN indicates chronic hypertension, DBP, diastolic blood pressure; and SBP systolic blood pressure. Reprinted with permission from Burwick et al.20 Copyright © 2013, American Heart Association, Inc.

*Controls matched 1:1 to cases by gestational age and parity. ¶P=0.0001, preeclampsia vs healthy controls or CHTN.
†P<0.001, CHTN vs healthy controls.
‡Data are medians (interquartile range).
§P=0.002, CHTN vs healthy controls.
||Wilcoxon signed-rank test P=0.01, preeclampsia vs healthy controls or CHTN.
**P=0.004, CHTN vs healthy controls.
**P=0.04, preeclampsia vs healthy controls.
function like macrophages to remove apoptotic debris from locally damaged tissue. Pertinent to our investigation, in mouse models of renal ischemia, upregulation of KIM-1 in the proximal tubule is specifically mediated by products of complement activation. Remarkably, and consistent with these data, we find that activated complement components show a strong positive correlation with increased urinary KIM-1 levels in human preeclampsia. Similar to mouse models, we also find that markers of terminal complement activation (C5a and C5b-9) rather than upstream activation (C3a) correlate most strongly with KIM-1 levels.

The association between KIM-1 and complement activation in severe preeclampsia is also strengthened by the lack of, or modest, association between complement components and other kidney injury markers in our study. Changes in the urinary excretion of B2M, cystatin C, osteopontin, and uromodulin have been associated with varying forms of kidney injury, but were not different between preeclampsia cases and controls. Increased urine NGAL has been associated with acute kidney injury, but we found levels to be increased in preeclampsia. EGF has been shown to be decreased in acute renal failure, obstructive nephropathy, and diabetic nephropathy, and we also found decreased urinary levels in preeclampsia. However, decreased excretion of both NGAL and EGF in preeclampsia was independent of complement components and may be influenced by alternative pathways of disease.

Our results suggest that in severe preeclampsia complement activation in the kidney is associated with a localized proximal tubule insult in association with KIM-1. Complement activation likely begins early in pregnancy with local effects at the placental interface, but may ultimately become a systemic process because of shedding of apoptotic or aneuploidy fetoplacental debris into the maternal circulation with resulting systemic inflammation and endothelial activation. Complement proteins may be filtered through an injured glomerulus and basement membrane or may propagate terminal complement activation locally at the proximal tubule, contributing directly to tubular inflammation (C3a, C5a) and cell death (C5b-9). A role for C5 activation in tubular injury is also supported by the finding that C5a receptors in the nephron are concentrated in the proximal tubule and that urinary excretion of C5b-9 correlates with active disease in severe preeclampsia. Considering our finding that terminal complement activation (ie, C5a and C5b-9) in severe preeclampsia is most closely associated with KIM-1, a specific marker of proximal tubule injury, targeted therapeutics to reduce complement activation or proximal tubule injury in severe preeclampsia may be useful in prevention or treatment of disease.

Perspectives
Our findings support the hypothesis that both glomerular damage and proximal tubule injury are central features of disease in severe preeclampsia. Furthermore, it seems that terminal complement activation at the level of C5, generating C5a and C5b-9, may propagate proximal tubule injury in active disease in association with KIM-1. Other measured urinary biomarkers of kidney injury do not seem to be specific for complement-specific effects in preeclampsia, although urinary NGAL and EGF excretion may be decreased in preeclampsia through other pathways. Because of the observational study design, we cannot be certain whether complement activation and proximal tubule injury are simply the final end points of severe disease in preeclampsia or whether such mechanisms are in effect before overt disease. The study is also limited by a small sample size that is underpowered to detect more modest effects between groups and limitations imparted by assessment of urine protein measurements from a single voided specimen. However, it is intriguing to note that complement blockade (at the level of C5) was able to prolong pregnancy for 17 days in a recently reported case of severe preeclampsia. Complement blockade has also been reported to restore kidney function in other severe medical conditions such as atypical hemolytic uremic syndrome, shiga toxin–associated hemolytic uremic syndrome, and catastrophic antiphospholipid antibody syndrome. Severe preeclampsia remains an unpredictable and fulminant disease, but we think there is a compelling rationale to target complement-mediated inflammation and proximal tubule injury in the treatment of preeclampsia.

Disclosures
Presented at the Society for Maternal-Fetal Medicine Annual Meeting, February 3–8, 2014, New Orleans, LA.
References


Rampersad R, Barton H. Hypertension 2013;14:270.


What Is New?

Kidney injury in severe preeclampsia is characterized by glomerular impairment, proximal tubule injury, and complement-mediated inflammation.

To severe preeclampsia, terminal complement activation is closely associated with kidney injury molecule-1, a specific marker of proximal tubule injury.

What Is Relevant?

Better understanding of glomerular and tubular patterns of kidney injury in severe preeclampsia may provide insights into disease management and treatment.

Targeted therapeutics to reduce complement activation and proximal tubule injury in severe preeclampsia may be useful in prevention or treatment of disease.

Summary

Using a comprehensive panel of urinary biomarkers, we describe a pattern of kidney injury in severe preeclampsia that is characterized by glomerular impairment, proximal tubule injury, and complement-mediated inflammation. Terminal complement markers, C5a and C5b-9, correlate most closely with urinary excretion of kidney injury molecule-1, suggesting that the effects of complement activation in severe preeclampsia may specifically localize to the proximal tubule. Complement blockade is an intriguing therapeutic option to reduce inflammation and kidney injury in severe preeclampsia.

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Title:  COMPLEMENT ACTIVATION AND KIM-1 ASSOCIATED PROXIMAL TUBULE INJURY IN SEVERE PREECLAMPSIA

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Short Title:  Complement Mediated Kidney Injury in Preeclampsia

Word Count:  Manuscript 5885 words, Abstract 244 words, 3 figures

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Email: burwick@ohsu.edu
S1. Kidney markers adjusted for urine creatinine in preeclampsia cases and controls.

<table>
<thead>
<tr>
<th>Urine Marker</th>
<th>Healthy Controls (n=25)</th>
<th>Chronic Hypertension (n=25)</th>
<th>Severe Preeclampsia (n=25)</th>
<th>p-value (PE vs. healthy controls)</th>
<th>p-value (PE vs. CHTN controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (ug/mg)</td>
<td>5.7 (3.4-11.9)</td>
<td>7.9 (5.8-15.2)</td>
<td>449 (151-1164)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Beta 2 microglobulin</td>
<td>483 (255-1025)</td>
<td>496 (294-1318)</td>
<td>463 (268-913)</td>
<td>0.86</td>
<td>0.58</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>42.9 (29.5-53.7)</td>
<td>70.8 (54.0-92.4)</td>
<td>52.8 (32.5-84.8)</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Epithelial growth factor</td>
<td>15.3 (11.8-18.3)</td>
<td>15.3 (10.9-20.6)</td>
<td>10.0 (8.2-12.2)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>KIM-1</td>
<td>0.62 (0.27-0.76)</td>
<td>0.51 (0.21-0.82)</td>
<td>1.60 (0.71-3.4)</td>
<td>0.002</td>
<td>0.01</td>
</tr>
<tr>
<td>NGAL</td>
<td>165 (50.2-210)</td>
<td>119 (46-267)</td>
<td>45.7 (16.2-128)</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>917 (519-1435)</td>
<td>1302 (1046-2048)</td>
<td>1406 (749-2124)</td>
<td>0.12</td>
<td>0.77</td>
</tr>
<tr>
<td>Uromodulin</td>
<td>9230 (3024-18410)</td>
<td>8580 (5882-11125)</td>
<td>3560 (2062-20476)</td>
<td>0.17</td>
<td>0.09</td>
</tr>
</tbody>
</table>
CHTN, chronic hypertension; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin; PE, preeclampsia
### S 2. Correlations between urine complement and kidney markers among severe preeclampsia subjects only (n=25)

<table>
<thead>
<tr>
<th>Urine marker</th>
<th>C3a*</th>
<th>p-value †</th>
<th>C5a*</th>
<th>p-value †</th>
<th>C5b-9*</th>
<th>p-value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.49</td>
<td>0.02</td>
<td>0.37</td>
<td>0.08</td>
<td>0.46</td>
<td>0.02</td>
</tr>
<tr>
<td>Beta 2 microglobulin</td>
<td>0.48</td>
<td>0.02</td>
<td>0.33</td>
<td>0.12</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.21</td>
<td>0.31</td>
<td>0.51</td>
<td>0.01</td>
<td>0.46</td>
<td>0.02</td>
</tr>
<tr>
<td>Epithelial growth factor</td>
<td>-0.01</td>
<td>0.98</td>
<td>0.27</td>
<td>0.17</td>
<td>0.16</td>
<td>0.43</td>
</tr>
<tr>
<td>KIM-1</td>
<td>0.17</td>
<td>0.43</td>
<td>0.60</td>
<td>0.001</td>
<td>0.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NGAL</td>
<td>0.20</td>
<td>0.34</td>
<td>0.12</td>
<td>0.58</td>
<td>0.29</td>
<td>0.17</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>-0.15</td>
<td>0.49</td>
<td>0.17</td>
<td>0.43</td>
<td>0.33</td>
<td>0.12</td>
</tr>
<tr>
<td>Uromodulin</td>
<td>0.05</td>
<td>0.82</td>
<td>-0.07</td>
<td>0.74</td>
<td>-0.07</td>
<td>0.76</td>
</tr>
</tbody>
</table>

KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin*

Spearman’s rho correlation coefficient

† Statistical significance at p=0.01 after Bonferroni correction for multiple comparisons