Messenger RNA Expression of Podocyte-Associated Molecules in Urinary Sediment of Patients with Lupus Nephritis

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ABSTRACT. Objective. To examine urinary expression of podocyte-associated molecules in patients with lupus nephritis (LN).

Methods. We studied 32 patients with active LN (Active group) and 17 patients with inactive lupus (Silent group). Messenger RNA expression of nephrin, podocin, and synaptopodin in urinary sediment was quantified by real-time polymerase chain reaction and compared to other clinical measures.

Results. The urinary concentrations of nephrin, podocin, and synaptopodin were significantly higher in the Active than the Silent group (p < 0.05 for all comparisons). There was no relation between urinary gene expression and the histological class of LN, but urinary nephrin expression correlated with proteinuria (r = 0.480, p < 0.01) and the score of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI; r = 0.578, p < 0.01). Urinary podocin expression also correlated with SLEDAI score (r = 0.389, p = 0.006). After initiation of immunosuppressive treatment, all patients were followed for an average of 13.7 ± 2.4 months. The decline of the glomerular filtration rate (GFR) correlated with urinary expression of podocin (r = 0.406, p = 0.005) and synaptopodin (r = 0.337, p = 0.021). In a multiple linear regression model, urinary podocin expression and baseline GFR were independent predictors of GFR decline.

Conclusion. The concentration of podocyte-associated molecules in urinary sediment correlated with lupus activity and GFR decline. The clinical utility of quantifying urinary expression of podocyte-associated molecules for risk stratification of patients with LN deserves further study. (First Release Nov 1 2007; J Rheumatol 2007;34:2358–64)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOUS NEPHRITIS
URINALYSIS
PODOCYTE-ASSOCIATED MOLECULES

Lupus nephritis (LN) is one of the most serious clinical manifestations of systemic lupus erythematosus (SLE). It is generally believed that development of renal injury in SLE originates from the complex immune responses triggered by interactions between autoantibodies and renal antigens. However, the exact immunopathogenesis of LN is still elusive. Recent studies show that podocyte injury occurs early in LN after deposition of immune complex, preceding the subsequent glomerular damage. Other research also shows that podocyte loss is a marker of disease activity in LN, which can be reversed by immunosuppressive therapy. In addition to podocyte loss, the expression pattern of podocyte-associated molecules, such as nephrin, podocin, and podoplanin, in patients with LN is significantly different from that of healthy controls. Therefore, detection of podocyte and associated molecular changes may be a useful way to assess the severity of LN.

Traditionally, renal biopsy is the standard method to study podocyte changes. Recently, urinary podocyte counting has been used as a noninvasive method in some diseases. However, these studies used sophisticated immunofluorescence methods for podocyte examination and had limited clinical applicability. We hypothesize that quantification of messenger RNA expression of podocyte-specific genes in urine sediment by reverse transcription and real-time quantitative polymerase chain reaction (PCR) may be a valuable noninvasive method for studying LN and other kidney diseases.
MATERIALS AND METHODS

Patients. We recruited 32 consecutive patients with active LN who required renal biopsy (the Active group) and 17 patients with silent lupus (the Silent group). All patients were diagnosed according to the American College of Rheumatology diagnostic criteria.

Morphometric study of kidney biopsy. Jones’ silver staining was performed on 5-µm thick sections of renal biopsy specimen. As described, we used a computerized image analysis method to semiquantitatively score glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis. Each lesion is scored 0 to 3 and the maximum is 12 points. Evaluation of biopsy specimens was performed by a single pathologist.

RESULTS

The demographic and baseline clinical data of the study subjects are summarized in Table 1. Renal biopsy of the Active group showed pure mesangial disease (class II, 2 cases), pure crescents, tubular atrophy, and interstitial fibrosis. Each lesion is scored 0 to 3 and the maximum is 12 points. Evaluation of biopsy specimens was performed by a single pathologist.

Table 1. Demographic and baseline clinical data.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Active Group</th>
<th>Silent Group</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F:M)</td>
<td>26:6</td>
<td>16:1</td>
<td>—</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>37.0 ± 10.9</td>
<td>40.2 ± 8.0</td>
<td>p = 0.19</td>
</tr>
<tr>
<td>Serum creatinine, µmol/l</td>
<td>116.6 ± 103.8</td>
<td>103.9 ± 72.7</td>
<td>p = 1.00</td>
</tr>
<tr>
<td>Urine protein, g/day</td>
<td>2.94 ± 2.06</td>
<td>0.64 ± 1.23</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Estimated GFR, ml/min</td>
<td>67.9 ± 39.6</td>
<td>70.7 ± 26.0</td>
<td>p = 0.60</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>12.6 ± 5.5</td>
<td>0.6 ± 0.9</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

GFR: glomerular filtration rate; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. * Data compared by Mann-Whitney U-test.

Statistical analysis. Statistical analysis was performed by SPSS for Windows software, version 11.0 (SPSS Inc., Chicago, IL, USA). All the results are presented as mean ± SD unless otherwise specified. Since data of target gene expression levels were highly skewed, either log-transformation or nonparametric statistical method was used. We used Mann-Whitney U-test to compare gene expression levels between groups and Spearman’s rank-order correlations to test associations between untransformed gene expression levels and clinical measures. Since the distribution of gene expression data of the Active and Silent groups became normal after log-transformation, Student’s t-test was used to compare the 2 groups. A p value < 0.05 was considered statistically significant. All probabilities were 2-tailed. In this series, we used 40 real-time quantitative PCR cycles as the detection limit of any target gene expression. To allow log-transformation of expression data, an expression value equal to half the detection limit (i.e., 41 cycles) was assigned when no detectable level of a transcript was found at 40 cycles, as recommended by Hulsken.

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membranous disease (class V, 9 cases), pure proliferative glomerulonephritis (class III or IV, 8 cases), and mixed proliferative and membranous disease (11 cases); 2 cases had insufficient material for histological classification. Histological activity and chronicity indices of the Active group were 7.0 ± 4.8 and 2.5 ± 2.0, respectively. Morphometric study showed that percentages of scarred glomerular and tubulointerstitial area were 13.56 ± 9.22% and 10.26 ± 3.83%, respectively.

Comparison of gene expression between groups. The concentrations of nephrin, podocin, and synaptopodin in the Active and Silent groups are summarized in Figure 1A. Urinary mRNA expression of nephrin, podocin, and synaptopodin was significantly higher in the Active than the Silent Group (Student’s t test, p < 0.01 for nephrin and podocin, p = 0.024 for synaptopodin). After adjustment for synaptopodin, gene expression of nephrin and podocin was still significantly higher in the Active group (p < 0.01 for both) (details not shown). There was no difference in the expression levels of urinary nephrin, podocin, and synaptopodin between patients with membranous (class V) and proliferative (class III or IV) nephritis (Figure 1B).

Relation with baseline clinical measures. The relation between urinary mRNA expression of podocyte-associated molecules and baseline clinical measures is displayed in Figure 2. Urinary nephrin expression correlated significantly with SLEDAI (r = 0.578, p < 0.01) and proteinuria (r = 0.480, p < 0.01), but not with GFR (r = 0.013, p = 0.9). Urinary podocin expression correlated significantly with SLEDAI (r = 0.389, p = 0.006), but not with proteinuria (r = 0.203, p = 0.162) or GFR (r = 0.014, p = 0.9).

Relation with renal histology. The correlation between urinary mRNA expression of podocyte-associated molecules and the degree of histological damage as quantified by morphometric study is summarized in Table 2. There was no significant correlation between urinary gene expression and the degree of glomerular and tubulointerstitial scarring. In addition, there was no correlation between urinary mRNA expression of podocyte-associated molecules and histological and chronicity index.

Relation with treatment response and renal function decline. The patients were followed for an average of 13.7 ± 2.4 months. After the initial induction treatment, 12 patients in the Active group had complete remission, 8 had partial remission, and 7 showed no response. Therapeutic response could not be determined in 5 patients because of inactive class II disease at diagnosis (2 cases), insufficient material for histologic diagnosis (2 cases), and loss to followup (1 case). There was no significant difference in urinary gene expression levels of target genes among patients with complete remission, partial remission, and no response.
The average rate of GFR decline after the initial induction phase of immunosuppressive therapy was –0.54 ± 2.19 ml/min/month. The rate of GFR decline correlated with the urine expression of podocin (r = 0.406, p = 0.005) and synaptopodin (r = 0.337, p = 0.021) (Figure 3). With a multiple linear regression model to adjust for the baseline SLEDAI, GFR, proteinuria, and histological measures, we found that urinary podocin concentration and baseline GFR were independent predictors of GFR decline (Table 3).

**DISCUSSION**

LN remains an important cause of mortality and morbidity in patients with SLE. Ideal markers for risk stratification and disease monitoring are still lacking. Recent studies showed that urinary mRNA expression of cytokines and growth factors may be a valuable noninvasive method for assessment of activity and severity of LN. However, quantification of urinary cytokine gene expression is not always reliable because it is prone to contamination with inflammatory cells.
from bladder origin, as well as gene expression by noninflammatory cells. We measured the mRNA of podocyte-associated molecules in urinary sediment because these target genes are specifically expressed in podocytes. In addition, podocyte injury is now recognized as a key step in the pathogenesis of LN as well as other primary glomerulonephritis.

In our study, all patients from both the Active and Silent groups had detectable urinary mRNA expression of podocyte-associated molecules. Indeed, all healthy controls also had detectable urinary expression of these molecules, albeit at a much lower level. We found higher levels of podocyte-associated molecules in the urinary sediment of the Active Group than in that of the Silent Group. In theory, the increase in gene expression may reflect either more podocytes are present in the urine or an upregulation of podocyte-associated molecule expression per podocyte. Besides the absolute expression level, which reflects the overall quantity of gene expression in urine sediment, we also normalize nephrin and podocin expression by that of synaptopodin, a method that is often used to control for the expression of other podocyte-associated molecules.15,16 Since the urinary expressions of both nephrin and podocin of the Active group remained higher than those in the Silent group after adjusting for synaptopodin expression, our result suggests an increase in urinary podocyte loss as well as an upregulation of the corresponding mRNA levels per podocyte in patients with active LN. The reason for nephrin and podocin upregulation in urinary podocytes is unknown. Clement, et al27 recently reported a downregulation of nephrin in intraglomerular podocytes in experimental nephrotic syndromes, suggesting that variation in podocyte gene expression profile is related to its destination—loss in urine or retention in the diseased glomeruli. It is, however, important to note that synaptopodin mRNA and protein levels do change in proteinuric diseases, and therefore the gene expression of synaptopodin cannot be regarded as a simple “housekeeping gene.”

Significant correlations are observed between urinary expression of podocyte associated molecules and SLEDAI, the degree of proteinuria, and baseline GFR. The correlation coefficients remain similar when the calculations are done with log-transformed values (details not shown). Of these relations, the correlation with SLEDAI is intriguing because this index is an assessment of overall disease activity rather than limited to kidney involvement. Our results suggest that measurement of urinary expression of podocyte-associated molecules in LN reflects lupus activity.

In our study, all active patients received standard immunosuppressive treatment. However, baseline gene expression level of urinary podocyte-associated molecules apparently did not predict therapy response. When patients with proliferative and membranous lesions were analyzed separately, there was no difference in baseline urinary gene expression for patients

Table 2. Correlation between urinary mRNA expression of podocyte-associated molecules and the degree of histological damage.

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Nephrin</th>
<th>Podocin</th>
<th>Synaptopodin</th>
</tr>
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<tbody>
<tr>
<td>Activity index</td>
<td>r = 0.112 p = 0.577</td>
<td>r = -0.014 p = 0.946</td>
<td>r = 0.233 p = 0.241</td>
</tr>
<tr>
<td>Chronicity index</td>
<td>r = -0.039 p = 0.084</td>
<td>r = 0.114 p = 0.946</td>
<td>r = 0.173 p = 0.388</td>
</tr>
<tr>
<td>Glomerular scarring</td>
<td>r = -0.019 p = 0.916</td>
<td>r = -0.053 p = 0.772</td>
<td>r = 0.162 p = 0.376</td>
</tr>
<tr>
<td>Tubulointerstitial scarring</td>
<td>r = 0.027 p = 0.884</td>
<td>r = 0.134 p = 0.465</td>
<td>r = 0.194 p = 0.287</td>
</tr>
</tbody>
</table>

Data are correlated by Spearman’s correlation.

Table 3. Multiple linear regression model of the rate of change of GFR.

<table>
<thead>
<tr>
<th>Unstandardized B</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.806</td>
<td>-0.542 to 4.154</td>
</tr>
<tr>
<td>Podocin</td>
<td>0.016</td>
<td>0.004 to 0.027</td>
</tr>
<tr>
<td>GFR</td>
<td>-0.033</td>
<td>-0.008 to -0.058</td>
</tr>
</tbody>
</table>

Figure 3. Relations between mRNA expression of podocyte-associated molecules in urinary sediment and the rate of change of estimated glomerular filtration rate (GFR), which reflects the severity of chronic progressive renal damage. A positive value of GFR change indicates improvement in renal function. Data compared by Spearman’s correlation coefficient.
with and without response to treatment in either group (details not shown), although the number of cases in each group was small. The urinary expression of podocyte-associated molecules correlates with the rate of decline of renal function, but not the histological chronicity index or glomerular or tubulointerstitial scarring. This phenomenon can possibly be explained because patients with mild disease would have minimal podocyte loss, while patients with grossly scarred kidneys would not have much podocyte remaining for urinary loss. Our study does not have any data on the delayed change in urinary gene expression profile after immunosuppressive therapy. Previous studies, however, have shown that the number of urinary podocytes decreases with immunosuppressive therapy. Previous studies, however, have shown that the number of urinary podocytes decreases with immunosuppressive therapy.\textsuperscript{30,31} Further studies are needed to clarify the role of serial measurement of these markers, and the rate of urinary podocyte loss at different disease stages.

A considerable proportion of the patients in our study had membranous nephropathy (class V). The distribution of the histological type of LN appears to be different from that in other studies, including our previous reports\textsuperscript{32,33}. The reason for this discrepancy remains unknown, but is unlikely to be selection bias because consecutive cases with renal biopsy were recruited in our study. We found that patients with type V nephritis showed the same expression level of the target genes as those with other types of nephritis, probably because of the small sample size. This phenomenon could also be explained because urinary podocyte loss is also present in patients with proliferative LN\textsuperscript{34}. In addition, occult type V LN may coexist with proliferative LN histologically\textsuperscript{17}. We found that urinary expression of podocyte-associated molecules was elevated in patients with active LN. The degree of upregulation of urinary nephrin expression correlated with SLEDAI, proteinuria, and renal function, while urinary podocin expression correlated with the rate of decline of GFR after therapy. The clinical utility of quantifying urinary expression of podocyte-associated molecules for risk stratification of LN deserves further study.

REFERENCES


