Urinary Excretion of Interleukin-6 in Pediatric IgA Nephropathy Patients

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Abstract

Objective: Urinary excretion of interleukin-6 (U-IL6) has been reported to be elevated and to represent the disease activity in adult IgA nephropathy (IgAN). We investigated the significance and the utility of U-IL6 activity in pediatric IgAN patients.

Methods: We evaluated 55 pediatric IgAN patients (4–18 years; mean age, 10.7 years) and 53 healthy controls from 2007 to 2012. The U-IL6 concentrations (pg/mg creatinine) were estimated by ELISA at the time of renal biopsy and after 6 months of prednisolone therapy for IgAN. In addition, we evaluated the correlations between the U-IL6 level and clinicopathological parameters of IgAN. To elucidate the usefulness for early diagnosis of IgAN, we investigated U-IL6 in 45 asymptomatic hematuria (ASH) children who were diagnosed by school urine screening program.

Results: U-IL6 activity was significantly higher in IgAN patients than in healthy controls and ASH children (p<0.01). In addition, U-IL6 was significantly decreased after 6 months of prednisolone therapy (p<0.01). With regard to the clinicopathological parameters, U-IL6 activity was correlated with degree of proteinuria (p<0.01, r=0.72), hematuria (p<0.01, r=0.54), urinary podocyte score (p<0.01, r=0.59), mesangial cell proliferation (p<0.05), endocapillary proliferation (p<0.01), and crescent formation (p<0.05). Interestingly, five children who transited to IgAN from ASH during the observation period showed high U-IL6 levels (p<0.01).

Conclusions: The present results also suggest that U-IL6 represents the disease activity in pediatric IgAN patients. We consider that it is important to evaluate U-IL6 in patients with ASH detected by school urinary screening program for early detection and prevention of unrecognized progression of IgAN.

Keywords: Asymptomatic hematuria; IgA nephropathy; Interleukin-6; Prednisolone; School urinary screening program; Urine biomarker; Urinary podocyte

Short Summary

Similar to previous reports, urinary excretion of IL-6 (U-IL6) was found to represent the disease activity in pediatric IgAN patients. It may be useful to evaluate U-IL6 activity in patients with asymptomatic hematuria detected by school urinary screening program for early detection and prevention of unrecognized progression of IgAN.

Introduction

IgA nephropathy (IgAN) is currently the most common form of primary glomerulonephritis around the world [1,2]. Long-term follow-up studies have shown that the disease progresses to renal failure in 20-50% of adult patients over 20 years [3]. Although the prognosis of childhood IgAN is believed to be benign, a study of 241 Japanese pediatric IgAN patients showed that 11% of them exhibited end-stage renal failure within 15 years [4]. The glomerular lesions are characterized by immune deposits of mainly IgA1 in the mesangium and by mesangial cell proliferation and extracellular matrix expansion [5]. Recent reports have suggested that several cytokines and growth factors, which are produced locally in the kidney, are directly related to the extent of the histological changes [6,7].

Interleukin (IL)-6 is produced in vivo by monocytes/macrophages, neutrophils, and endothelial, mesangial, and epithelial cells [8,9]. IL-6 has been identified within the glomeruli of IgAN patients and contributes to the proliferation of mesangial cells [10]. Increased urinary excretion of IL-6 (U-IL6) has been reported in adult IgAN patients with a progressive clinical course [11-13]. To date, high U-IL6 has not been reported in pediatric IgAN patients.

In 1973, the Japanese Ministry of Education began a mass urine screening program for school children aimed at the early detection of insidious renal disease [14,15]. Children with both hematuria and proteinuria have been suspected to have serious glomerular diseases (mainly IgAN) and are indicated for a renal biopsy [15]. On the other hand, children with isolated asymptomatic hematuria (ASH) have been generally considered to have a good prognosis and are only indicated for a renal biopsy when they show proteinuria associated with hematuria [16]. Measurement of U-IL6 may be one of the useful markers for early detection of pediatric IgAN patients who initially show only ASH in the school urinary screening program.

In the present study, we measured the U-IL6 activity in pediatric IgAN patients and ASH children to demonstrate its potential association with the disease severity and its value as a urinary marker for early detection of IgAN.

Subjects and Methods

Patients

The investigation complied with the principles outlined in the Declaration of Helsinki. Our IRB/Ethics Committee decided that approval was not required for this study. In the study, we investigated 55 pediatric IgAN patients (31 males and 24 females; mean age: 10.5 ± 3.2 years) newly diagnosed with IgAN, 45 children with ASH (22

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From April 2007 to June 2012, a total of 55 Japanese children with IgAN (31 males and 24 females; average age: 10.5 ± 3.2 years) were admitted and underwent a renal biopsy. Among them, 51 patients received prednisolone therapy and two patients received angiotensin-converting enzyme inhibitors and dipyridamole. Table 1 show the clinical and laboratory findings of the IgAN patients at the renal biopsy and after 6 months of prednisolone therapy. Five patients (9.1%) exhibited nephrotic syndrome and one patient had renal insufficiency at the time of renal biopsy. Macrohematuria was observed in 17 patients (30.9%). The mean proteinuria was 1.41 ± 1.21 g/g Cr and the mean eGFR was 124 ± 42.1 mL/min/1.73 m² at the time of renal biopsy. Macrohematuria was observed in 17 patients (30.9%).

Table 1: Clinical characteristics and laboratory findings of the IgA nephropathy patients at the renal biopsy and after 6 months of prednisolone therapy.

<table>
<thead>
<tr>
<th>n</th>
<th>At Biopsy</th>
<th>6 months after PSL</th>
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<tbody>
<tr>
<td>SBP(mmHg)</td>
<td>116 ± 21</td>
<td>114 ± 19</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>68 ± 19</td>
<td>63 ± 18</td>
</tr>
<tr>
<td>eGFR(mL/min/1.73 m²)</td>
<td>124 ± 42.1</td>
<td>129 ± 36.3</td>
</tr>
<tr>
<td>Proteinuria(g/g Cr)</td>
<td>1.41 ± 1.21</td>
<td>0.22 ± 0.44 *</td>
</tr>
<tr>
<td>Microhematuria</td>
<td>17/55(30.9%)</td>
<td>0/51(0%)</td>
</tr>
<tr>
<td>RBC ≥ 50/hpf</td>
<td>21/55(38.2%)</td>
<td>2/51(3.9%)</td>
</tr>
<tr>
<td>20 ≤ RBC &lt; 50/hpf</td>
<td>11/55(20%)</td>
<td>9/51(17.6%)</td>
</tr>
<tr>
<td>RBC &lt; 20/hpf</td>
<td>6/55(12%)</td>
<td>40/51(78.4%)</td>
</tr>
<tr>
<td>Urinary podoocyte/μl</td>
<td>3.76 ± 1.82</td>
<td>0.85 ± 0.91 *</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>5/55(9.1%)</td>
<td>0/51(0%)</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>1/55(2%)</td>
<td>0/51(0%)</td>
</tr>
</tbody>
</table>

PSL: prednisolone; SBP: systolic blood pressure; DBP: diastolic blood pressure; hpf: high-power field; eGFR: estimated glomerular filtration rate; RBC: red blood cells.

*p<0.05
Urinary excretion of interleukin-6 in IgA nephropathy children

The IgAN patients at the time of the renal biopsy had significantly higher U-IL6 levels than the healthy controls (9.1 ± 9.13 vs. 1.5 ± 0.33 pg/mg Ucr, p<0.01, Figure 1). After 6 months of prednisolone therapy, the U-IL6 level was significantly reduced in the 51 IgAN patients (9.1 ± 9.13 to 1.78 ± 1.76 pg/mg Ucr, p<0.01, Figure 2).

Correlations of urinary excretion of interleukin-6 with clinical and histological findings

Table 2 shows the correlations of the U-IL6 level with the clinical and histological findings. In the clinical findings, the U-IL6 level of the IgAN patients at the renal biopsy showed positive correlations with the degree of proteinuria (r=0.72, p<0.01), hematuria (r=0.54, p<0.01), and U-Pod number (r=0.59, p<0.01). In contrast, the U-IL6 level had a negative correlation with the eGFR (r=-0.34, p=0.015). In the histological findings, the U-IL6 level of the IgAN patients at the renal biopsy showed positive correlations with the degree of mesangial hypercellularity (proliferation score 2–3 vs. 0–1, p=0.047), crescent formation (cellular or fibrocellular crescent positive vs. negative, p=0.042), and endocapillary proliferation (positive vs. negative, p<0.01). The percentages of mesangial proliferative glomeruli did not differ significantly (diffuse vs. focal, p>0.05).

<table>
<thead>
<tr>
<th>Clinical Findings</th>
<th>r</th>
<th>p</th>
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<tbody>
<tr>
<td>Proteinuria (g/g Cr)</td>
<td>0.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hematuria (score)</td>
<td>0.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urinary podocyte (ml)</td>
<td>0.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>eGFR (ml/min/m²)</td>
<td>-0.34</td>
<td>0.015</td>
</tr>
<tr>
<td>Histological Findings</td>
<td></td>
<td></td>
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<tr>
<td>Mesangial cell proliferation</td>
<td></td>
<td></td>
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<tr>
<td>2-3 (n=23) vs. 0-1 (n=32)</td>
<td></td>
<td>0.047</td>
</tr>
<tr>
<td>Mesangial proliferative glomeruli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diffuse(n=24) vs. focal(n=31)</td>
<td></td>
<td>NS</td>
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<tr>
<td>Cellular/fibrocellular crescent</td>
<td></td>
<td></td>
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<tr>
<td>positive(n=24) vs. negative(n=31)</td>
<td></td>
<td>0.042</td>
</tr>
<tr>
<td>Endocapillary proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive(n=29) vs. negative(n=26)</td>
<td></td>
<td>&lt;0.01</td>
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</tbody>
</table>

eGFR, estimated glomerular filtration rate; NS, not significant

Hematuria scores (red blood cells/high-power field): 0, <10; 1, ≥10 to <20; 2, ≥20 to <30; 3, ≥30 to <50; 4, ≥50 to <100; 5, ≥100

Table 2: Correlations of urinary excretion of interleukin-6 with clinical and histological findings.

Urinary excretion of interleukin-6 in asymptomatic hematuria children

Figure 3 shows the U-IL6 levels at the renal biopsy of the IgA nephropathy (IgAN) patients, asymptomatic hematuria children, and controls. NS: not significant.

p<0.01. The percentages of mesangial proliferative glomeruli did not differ significantly (diffuse vs. focal, p>0.05).

Urinary excretion of interleukin-6 in asymptomatic hematuria children

Figure 3 shows the U-IL6 levels at the renal biopsy of the IgA patients and ASH children. The U-IL6 levels were significantly lower in the 45 ASH children diagnosed by the urine screening program than in the IgAN patients (1.96 ± 1.3 vs. 9.1 ± 9.13 pg/mg Ucr, p<0.01 by ANOVA). On the other hand, the U-IL6 levels in the ASH children did not differ significantly from those in the healthy controls (1.96 ± 1.3 vs. 1.5 ± 0.33 pg/mg Ucr, p>0.05 by ANOVA). Among the 45 ASH children, three males and two females transitioned to IgAN during the observation period. Table 3 shows the clinical and laboratory findings of these five children who transitioned from ASH to IgAN. At the school urine screening tests, all five children showed only microhematuria and did not have positive proteinuria. In addition, their U-pod numbers remained within the normal limit (<1.0/mL). However, the U-IL6 levels
in these five children were significantly higher than those in the other 41 ASH children (4.3 ± 1.18 vs. 1.61 ± 1.17 pg/mg Ucr, p<0.05). At IgAN onset, all five children showed positive proteinuria and increased U-pod numbers (4.81 ± 3.5/mL). Their U-IL6 levels tended to increase steadily with the onset of IgAN (4.3 ± 1.18 to 6.26 ± 1.67 pg/mg Ucr, respectively) in the disease course in Japan. On the other hand, the prognosis of children with ASH is considered to be good, and a renal biopsy is only indicated for children with ASH who also show proteinuria [16]. Hisano et al. [16] reported that seven of 136 ASH children were diagnosed with IgAN by a renal biopsy because of evidence for proteinuria or macrohematuria throughout the follow-up period. Among these seven IgAN children, one child exhibited renal insufficiency at the renal biopsy and developed persistent proteinuria. Hence, we think that a more benign prognosis of IgAN may be approved by the identification and validation of urinary markers that can distinguish the presence of IgAN from ASH in children.

The scoring of U-pods has been reported to be one of the useful clinical diagnostic tools for the acute state of IgAN and Henoch–Schönlein purpura nephritis [18]. The present study also showed that higher U-pod numbers were found in pediatric IgAN patients. In contrast, all the ASH children, including the five patients who transited to IgAN during the observation period, showed normal U-pod numbers. Therefore, we consider that scoring of U-pods is not a suitable marker for early distinction of IgAN from ASH. In the present study, the five IgAN children who progressed from ASH showed higher U-IL6 numbers. Thus, the scoring of U-pods may be the useful markers for early detection and prevention of unrecognized progression of IgAN.

In summary, the present results suggest that higher U-IL6 reflects the disease severity in pediatric IgAN patients. In addition, the significant reduction in U-IL6 after prednisolone therapy may represent a suitable marker of the disease activity. We propose that it is important to evaluate U-IL6 in patients with ASH detected by the school urinary screening program for early detection and prevention of unrecognized progression of IgAN. However, further studies are required to elucidate whether U-IL6 measurements can be used as a noninvasive tool to select children who may need more intensive examinations including a renal biopsy.

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**References**


