Clinical and Histological Characteristics in Patients with Non–IgA Mesangioproliferative Glomerulonephritis

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Background: Mesangioproliferative glomerulonephritis is the most common type of chronic glomerulonephritis (CGN). However, the clinical characteristics and prognosis are not fully understood in patients without Immunoglobulin A (IgA) deposition. To explore the clinical and pathological characteristics of patients with mesangioproliferative glomerulonephritis without IgA deposition (N–IgAN), we performed dual retrospective analyses.

Methods: A single-center study was performed in 60 patients with biopsy-proven N–IgAN. 98 age- and sex-matched IgA nephropathy (IgAN) patients were randomly selected as a control group. The clinical and histopathological data at the time of renal biopsy were compared between N–IgAN and IgAN. In a second study, the data for 477 patients who had undergone maintenance renal replacement therapy (RRT) was collected and examined for the causal primary diseases.

Results: Duration from onset of renal symptoms to renal biopsy in patients with N–IgAN (71.2 ± 123.3 months) was significantly longer than that in patients with IgAN (65.9 ± 74.9 months) (p=0.0328). Urinary protein excretion in N–IgAN patients (0.6±1.1 g/gCr) was significantly lower than that in IgAN (1.0 ± 1.3 g/gCr) (p < 0.0001). Ratio of global sclerosis, segmental sclerosis, crescents, interstitial mononuclear cell infiltration, interstitial fibrosis, and tubular atrophy were significantly lower in N–IgAN patients. Of the 477 patients who had undergone maintenance RRT, 95 patients had CGN (19.9%). Among them, 37 patients had received a renal biopsy, only one patient was N–IgAN (1%).

Conclusion: It appears that N–IgAN can be recognized as a benign disease entity in comparison with IgAN.

Key words: non–IgA mesangioproliferative glomerulonephritis, IgA nephropathy, end stage renal desease (ESRD)

Introduction

Mesangioproliferative glomerulonephritis describes a renal specimen that presents mesangial hypercellularity and/or an increase in the mesangial matrix. IgA nephropathy (IgAN) is characterized by mesangioproliferative glomerulonephritis with IgA deposits and is recognized as the most common primary glomerular disease worldwide. The clinical manifestations and long-term outcome of IgAN are well defined. In contrast, for patients with mesangioproliferative glomerulonephritis without IgA deposition (N–IgAN), there are few reports describing the clinical characteristics and prognosis. According to previous reports, N–IgAN presents the histological features of mesangioproliferative glomerulonephritis without IgA deposits by immunofluorescence and no electron dense deposits (EDD) in the paramesangial areas by electron microscopic observation. The Figure–I shows the representative histological findings of IgAN and N–IgAN. In nationwide biopsy registry data from Japan, N–IgAN comprises 3.5% of all biopsy–proven renal diseases. However, the prevalence of this disease varies country to country.

A retrospective study from Norway reported that the progression rate to end stage renal disease (ESRD) was similar for both IgAN and N–IgAN.
In 2010, Owada et al. from Japan reported that the renal outcome of 84 N-IgAN patients with no medication was stable at end of the follow up during 3.25 ± 1.91 years. The authors concluded that N-IgAN is a benign entity\(^3\). However, more recently a study from India came to a significantly different conclusion\(^4\). Waikhom et al. reported that most patients developed chronic kidney disease (CKD), stage 3 or over, despite medication. The authors concluded that N-IgAN is not a benign disease and is associated with ESRD\(^4\).

To clarify these discrepancies, we strictly diagnosed N-IgAN by light microscopy, immunofluorescence, and electron microscopy, and compared the patients of N-IgAN with those of age- and sex-matched IgAN. The comparison was performed from the view of the clinical profile, histopathological variables, and long-term outcome.

### Materials and Methods

**1. Clinical characteristics at the time of renal biopsy**

This study was conducted at the Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan. From April 1977 to October 2013, a total of 1,859 biopsies were performed at the Juntendo University Hospital. Of these, 876 patients (47.1%) were diagnosed as IgAN and 60 patients (3.2%) were diagnosed as N-IgAN, with features of mesangial hypercellularity and/or an increase in the mesangial matrix on light microscopy without IgA deposits by immunofluorescence, with no EDD by electron microscopic observation. Since the precision is increased the ratio of controls to cases\(^10\), 98 sex- and age-matched patients were randomly selected from the IgAN group as a control. Data at the time of renal biopsy were compared between N-IgAN group and IgAN group.
group. The levels of protein, red blood cells, N-acetyl-β-D-glucosaminidase and β2-microglobulin in urinary samples, 24 hours creatinine clearance, and the estimated glomerular filtration rate (eGFR) were collected. Red blood cells, hemoglobin, hematocrit, platelet, total protein, albumin, serum urea nitrogen (SUN), serum creatinine (s-Cr), uric acid (UA), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglyceride (TG), IgG, IgA, IgM, C3, C4, and total complement hemolytic activity (CH50) were examined. Systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), the interval from onset to the renal biopsy, and the pathological findings were also examined. Hematuria was graded as follows: grade 1, 1-5/high power field (HPF); grade 2, 6-10/HPF; grade 3, 11-15/HPF; grade 4, 16-20/HPF; and grade 5, > 21/HPF.

In the light microscopic study, the renal tissues were stained with hematoxylin and eosin, periodic acid-Schiff, Azan, and periodic acid-methenamine-silver. We defined mesangioproliferative glomerulonephritis as being four or more cells per mesangial area. Fluorescein isothiocyanate-labeled antisera to human IgG, IgM, IgA, C3c, and C1q were used for the immunofluorescent procedure. In the electron microscopic study, one or two glomeruli were observed in each patient. These glomeruli were observed for the location of deposits and the partial thinning of glomerular basement membrane (GBM). Histological findings were evaluated by at least two pathologists and two nephrologists. The Figure-1 shows the representative histological findings of IgAN and N-IgAN. The study was approved by the institutional review board of Juntendo University Hospital, and all patients gave the written informed consent.

2. Retrospective analysis of end stage renal disease (ESRD)

In our hospital, 477 patients underwent maintenance renal replacement therapy (RRT), including hemodialysis (HD) or peritoneal dialysis (PD) from 2008 to 2012. We collected their medical histories and then explored their original diseases of ESRD.

3. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5J for Windows (GraphPad, San Diego, CA, USA). Most data were presented as mean ± standard deviation and some data were presented as proportions. As non-normal distributions or inequality of variances was present, nonparametric analysis was performed. Statistical comparisons were performed using the Mann-Whitney’s U test, Chi-square test, and Fisher’s exact test. A p-value of < 0.05 was considered statistically significant.

Results

1. Clinical parameters

Clinical characteristics of patients with N-IgAN and those with IgAN at the time of renal biopsy are shown in Table-1. The mean age at onset and gender presented no difference between N-IgAN and IgAN. There were no significant differences in the BMI between N-IgAN and IgAN. However, the duration from onset to renal biopsy in patients with N-IgAN (71.2 ± 123.3 months) was significantly longer than that in patients with IgAN (65.9 ± 74.9 months) (p = 0.0323). SBP in patients with N-IgAN

<table>
<thead>
<tr>
<th>Patient (number)</th>
<th>N-IgAN 60</th>
<th>IgAN 98</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (number)</td>
<td>27/33</td>
<td>50/48</td>
<td>ns</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>36.5 ± 15.9</td>
<td>34.7 ± 13.5</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 3.5</td>
<td>22.6 ± 3.2</td>
<td>ns</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>121.5 ± 16.5</td>
<td>116.3 ± 12.9</td>
<td>0.0453</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70.8 ± 12.2</td>
<td>68.4 ± 10.8</td>
<td>ns</td>
</tr>
<tr>
<td>Interval from onset to renal biopsy (month)</td>
<td>71.2 ± 123.3</td>
<td>65.9 ± 74.9</td>
<td>0.0323</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ns, not significant, p < 0.05

Table-1 Comparison of the baseline clinical characteristics of patients with N-IgAN and those with IgAN at the time of biopsy
(121.5 ± 16.5 mg/dl) was significantly higher than that in patients with IgAN (116.3 ± 12.9 mg/dl) \((p = 0.0453)\). There were no significant differences in the DBP between N-IgAN and IgAN.

**2. Laboratory data**

Blood examinations in N-IgAN and IgAN patients at the time of renal biopsy are shown in Tables 2 and 3. At the time of renal biopsy, s-Cr was the same level (0.8 ± 0.3 mg/dl) in each group. SUN, eGFR, UA, T-Cho, LDL-C and TG and albumin presented no significant difference between the two groups.

The mean levels of serum IgA in patients with N-IgAN (250.0 ± 131.0 mg/dl) were significantly lower than those in patients with IgAN (318.1 ± 103.5 mg/dl) \((p = 0.0004)\). The mean levels of IgG and IgM presented no significant difference. CH50 in patients with N-IgAN (37.9 ± 9.5 mg/dl) was significantly lower than that in patients with IgAN (42.2 ± 7.7 mg/dl) \((p = 0.0026)\). The mean levels of C3 and C4 presented no significant difference. The levels of serum IgA/C3 ratio in patients with N-IgAN (2.7 ± 1.7) were significantly lower than that in patients with IgAN (3.1 ± 1.1) \((p = 0.0148)\).
3. Urinary examination

Although all patients showed hematuria in the two groups, the mean urinary blood cell grades were almost same between both groups (Table-2). Urinary protein excretion in patients with N-IgAN (0.6 ± 1.1 g/gCr) was significantly lower than that in patients with IgAN (1.0 ± 1.3 g/gCr) (p < 0.0001). Urinary N-acetyl-β-D-glucosaminidase in patients with N-IgAN (7.6 ± 6.3 IU/l) was significantly lower than that in patients with IgAN (11.8 ± 9.2 IU/l) (p = 0.0118). Urinary β2-microglobulin presented no significant difference.

4. Histopathological findings

The number of global sclerosis, segmental sclerosis, and crescents in patients with N-IgAN was significantly lower than that in patients with IgAN (p = 0.0140, p = 0.0059, p < 0.0001, respectively, Table-4). The number of patients who had arteriosclerosis presented no significant difference between N-IgAN and IgAN. The number of patients who had interstitial mononuclear cell infiltration and fibrosis were significantly lower in N-IgAN than IgAN (p < 0.0001, p = 0.0007, respectively). The number of patients who had tubular atrophy was significantly lower in N-IgAN than IgAN (p < 0.0001).

Immunofluorescence results showed that about one third of patients from both groups presented depositions of IgG, IgM and C1q. Patients who had C3c deposits in N-IgAN (22.4%) were significantly lower than those in IgAN (99.0%) (p < 0.0001).

In electron microscopic findings, the number of patients who had a partial thinning of GBM and foot process effacement was significantly lower in N-IgAN than IgAN (p < 0.0001, p < 0.0001, respectively).

5. Retrospective analysis of ESRD

A total of 477 ESRD patients underwent maintenance RRT. There were 341 men and 136 women (Figure-2). Among them, the dominant original renal diseases were diabetic nephropathy (180 patients, 37.7%), hypertensive nephrosclerosis (104 patients, 21.8%), and chronic glomerulonephritis (CGN) (95 patients, 19.9%). 37 out of 95 CGN patients (38.9%) had their original renal diseases confirmed by renal biopsy. 26 patients were IgAN (27.3%), 6 patients had membranous nephropathy (6.3%), 3 patients had focal segmental glomerular

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### Table 4 Comparison of the pathological findings at the time of renal biopsy

<table>
<thead>
<tr>
<th></th>
<th>N-IgAN</th>
<th>IgAN</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[Light microscopy]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of glomeruli</td>
<td>15.8 ± 8.1</td>
<td>16.1 ± 8.2</td>
<td>ns</td>
</tr>
<tr>
<td>Global sclerosis</td>
<td>1.0 ± 1.4</td>
<td>2.1 ± 2.8</td>
<td>0.0140</td>
</tr>
<tr>
<td>Segmental sclerosis</td>
<td>0.2 ± 0.6</td>
<td>0.9 ± 1.9</td>
<td>0.0059</td>
</tr>
<tr>
<td>Crescent</td>
<td>0.3 ± 1.0</td>
<td>1.3 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
<td>28.3%</td>
<td>33.7%</td>
<td>ns</td>
</tr>
<tr>
<td>Interstitial infiltration</td>
<td>38.3%</td>
<td>73.5%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>46.7%</td>
<td>72.4%</td>
<td>0.0007</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>41.7%</td>
<td>81.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>[Immunofluorescence]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>28.3%</td>
<td>37.8%</td>
<td>ns</td>
</tr>
<tr>
<td>IgM</td>
<td>25.0%</td>
<td>37.8%</td>
<td>ns</td>
</tr>
<tr>
<td>C1q</td>
<td>0%</td>
<td>0%</td>
<td>ns</td>
</tr>
<tr>
<td>C3c</td>
<td>22.4%</td>
<td>99.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>[Electron microscopy]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial GBM thinning</td>
<td>16.7%</td>
<td>49.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Foot process effacement</td>
<td>60.0%</td>
<td>87.8%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

IgG, immunoglobulin G; IgM, immunoglobulin M; GBM, glomerular basement membrane; ns, not significant
sclerosis (FSGS) (3.1%), and only one patient was N-IgAN (1%). This N-IgAN patient began HD at 64 years of age. His proteinuria was observed when he was 16 years old and gradually hematuria was also observed. When he was 23 years old, a renal biopsy was performed and he was diagnosed as N-IgAN. At the time of renal biopsy, his BMI was 19.6, blood pressure was 140/80 mmHg, hematuria was 5 RBCs/HPF, urinary protein excretion was 2.0-2.5 g/day, SUN was 25 mg/dl, and s-Cr was 0.8 mg/dl. Although angiotensin receptor type II blockers, calcium channel blockers, α blockers, and β blockers were used, his blood pressure was poorly controlled. His systolic blood pressure was usually over 150 mmHg. Since allopurinol was also used, his serum UA level was well controlled. As eicosapentaenoic acid ethyl ester and hydroxymethyl glutaryl-coenzyme a reductase inhibitors were used, his cholesterol was also well controlled, but his TG was poorly controlled. For the reduction of urinary protein, dilazep hydrochloride was used, but there was no efficacy, and urinary protein excretion was usually 2.0-2.5 g/day continuously. He did not receive any steroid and immunosuppressive drugs. His renal function gradually decreased and he began HD 48 years after onset.

Discussion

Among mesangioproliferative glomerulonephritis, IgAN is recognized as the most common form of primary glomerulonephritis worldwide. The spectrum of clinical presentation, treatment response, histopathological characteristics, and long-term outcome are well known. In contrast, the clinical
course and pathophysiology of N-IgAN has not been well established. We compared N-IgAN patients with randomly selected sex- and age-matched IgAN patients. In this study, their clinical symptoms, histopathological characteristics, and long-term outcome were better than IgAN. Previously, Owada et al.\(^3\) reported on the outcome of 84 N-IgAN patients, found that renal function was stable at end of a follow up period of 3.25 ± 1.91 years, and concluded that N-IgAN is a benign entity. Our study supports their conclusion. More recently, Waikhom et al. from India\(^4\) reported on the outcome of 57 N-IgAN patients followed up for 3 years, found that most of patients developed chronic kidney disease (CKD) of stage 3 or over despite medications, and concluded that N-IgAN is not a benign renal disease. However, 82.1% of their included patients were nephrotic syndrome patients\(^4\). The difference in results therefore might be associated with sample collection since our study enrolled only 2 cases of nephrotic syndrome. When we also analyzed the data didn’t excluding the patients with nephrotic syndrome, our results of the paper didn’t change.

N-IgAN and IgAN had common urinary findings, such as urinary blood cells and urinary protein excretion. But this study showed that urinary protein excretion in patients with N-IgAN was significantly lower than that in patients with IgAN. At the time of renal biopsy, renal function and the factors that cause arteriosclerosis presented no significant difference between the two groups. It is well known that proteinuria is a strong, independent predictor of ESRD\(^15,16\). SBP in patients with N-IgAN was significantly higher than that in patients with IgAN. Functional impairment of the kidney is more correlated with the degree of tubulointerstitial damage than that of glomerular injury\(^19\). It may affect the prognosis of N-IgAN and IgAN. In immunofluorescence, the ratio of patients who had depositions of IgG and/or IgM presented no significant difference, but the ratio of patients who had C3c deposits was significantly lower in N-IgAN than IgAN. C3 deposits represent that complement pathway activates in the local tissues. Thus, it may suggest that the immune-complex is not directly involved in the pathogenesis of N-IgAN.

Serum CH50 is a blood test that helps determine whether protein production, consumption and deficiency in the complement components. The levels of serum CH50 in N-IgAN patients was significantly lower than those in IgAN patients. In recent years, it has been proposed that the activation of the complement pathway in the renal tissues of IgA patients primarily mediates the alternative pathway (AP), as well as the lectin pathway (LP) in some patients. We have reported that both serum C4 and CH50 levels in IgAN patients were higher than those in healthy subjects\(^20\). Most patients with IgAN showed a chronic clinical course, and the production of complement components might be stimulated by persistent inflammation in the pathogenesis of IgAN. Suzuki et al\(^22\) measured the complement components in 122 patients with IgAN and compared them with the time of renal biopsy and last follow-up period. Focusing on the fluctuations of serum C3 levels, they found that the serum C3 levels in
levels of the patients were significantly increased at last observation, along with reduction of disease activity. Although the serum levels of complement components were the results of the production and consumption of complements, these mechanisms might not evident in patients with N-IgAN.

Most patients with CGN underwent long clinical courses. Since it is difficult to follow their clinical courses in single center, we studied the causes of ESRD. 37 out of 95 CGN patients had renal biopsy. 26 patients were IgAN (27.3%), and only one case was N-IgAN (1%). The one patient with N-IgAN initiated HD after 48 years after onset, taking a longer time to initiate RRT than in IgAN patients. Several drugs were needed to control his blood pressure, high UA, and lipid disorder. These clinical findings may have influenced his renal function.

Many surveys of IgAN have been undertaken, with IgAN leading to ESRD in 30–40% of patients within 20 years after diagnosis. In contrast to IgAN, it appears that N-IgAN is a disease that has a low risk of leading to progressive renal injury ultimately requiring RRT.

References