Changes of Glomerular Density in Childhood IgA Nephropathy

Yoshihiro Shimada

Department of Pediatrics, Hufüidera Municipal Hospital

Abstract

Background. IgA nephropathy is the most common types of chronic glomerulonephritis in Japan. Histologic prognostic factors include marked glomerulosclerosis and tubular interstitial abnormalities. Recent studies concluded that a low glomerular density at clinical onset influenced the prognosis of adults with IgA nephropathy. However, no similar relationship has been reported between the glomerular density and prognosis in children.

Methods. In 22 children diagnosed with IgA nephropathy showing an unfavorable prognosis based on renal biopsy specimen findings, we histologically compared the glomerular density and related variables before therapeutic intervention with the same variables after clinical remission.

Results. No significant differences were evident in creatinine clearance or the estimated glomerular filtration rate between the 2 time points. On the other hand, urinary protein, serum IgA, serum complement C3, and total serum cholesterol were significantly lower at the time of the second biopsy than at the first biopsy. The mean glomerular density was $6.9 \pm 2.2/\text{mm}^2$ in the first specimen and $7.0 \pm 2.4/\text{mm}^2$ in the second specimen. Although few children showed low glomerular density at either time point, a negative correlation was evident between the glomerular density in the first biopsy specimen and degree of change of glomerular density over time, as was reported in adults. Furthermore, negative correlations were found between the glomerular density in the second specimen and mean glomerular area, maximum glomerular area, and glomerular volume. Glomerular enlargement was noted in children with a low glomerular density.

Conclusions. No significant differences were evident in the glomerular density or its relationship to clinical prognosis between the 2 time points. However glomerular enlargement was shown in patients with low glomerular density. Kidney disease during childhood induces glomerular enlargement prior to adulthood, which can predict likelihood of be progression of IgA nephropathy even in children.

Key words: IgA nephropathy, glomerular density, glomerular enlargement, prognostic factors, children

Introduction

IgA nephropathy (IgAN) is the most common types of chronic glomerulonephritis in Japan. In patients with IgAN, adverse clinical predictors include severe proteinuria, hypertension at onset and during subsequent observation, and also elevated serum creatinine (s-Cr) at the onset. Important histologic predictors include marked glomerulosclerosis and tubular interstitial abnormalities. Combination therapy with steroids and immunosuppressive drugs has improved treatment responses; the prognosis associated with childhood IgAN is now more favorable than in adult IgAN. However, in a study involving 241 Japanese children, IgAN progressed to chronic renal failure in 11% over an observation period of 15 years; favorable long-term outcomes cannot be assumed. Several studies have correlated histologic features of IgAN in renal
biopsy specimens with clinical outcome. Glomeruli are formed before gestational week 34. Glomerular volume increases during growth via neogenesis of glomerular capillaries, but number of glomeruli and the glomerular density remain constant. Approximately 1000000 glomeruli are present in each kidney although some studies of autopsy kidneys reported substantial variation in glomerular number. In premature or malnourished infants, however, development of structures such as the central nervous system, eyes, lungs, and kidneys is delayed. Such functional and structural immaturity contributes importantly to onset of various diseases during child- or adulthood, as stated in the Developmental Origins of Health and Disease (DOHaD) theory. If the number of renal glomeruli is low at birth, glomerular density is decreased. Several studies identified low birth weight (LBW) as a risk factor for kidney diseases, including focal glomerulosclerosis. We have encountered children with severe chronic glomerulonephritis, including some with IgAN, whose creatinine clearance (Ccr) exceeded the normal range at presentation and remained high during outpatient follow-up after the initial lesions had subsided. Among these children, the mesangial proliferation evident in the initial specimen decreased, but the number of glomeruli per unit area was still reduced and glomerular enlargement persisted. Even when clinical remission is achieved in moderate or severe IgAN, patients who show histologic findings such as marked mesangial proliferation and crescent formation at onset may have fewer functioning glomeruli because of the presence of glomeruli with initial injury severe enough to preclude recovery. The result is overloading of residual glomeruli. Recent studies have linked decreases in the glomerular density with glomerular enlargement with a poor outcome in patients with chronic kidney diseases, including IgA and obesity-associated nephropathies. Factors such as glomerular density and diameter, which once were not considered predictive of outcome, now are thought to be associated with the long-term prognosis for patients with chronic kidney disease. However, few studies have reported the histologic assessment including these factors in childhood IgAN. This study considers possible associations of histologic parameters such as glomerular density and glomerular area with clinical features in children with IgAN.

Methods

Selection of subjects
Subjects were 22 children (16 boys, 6 girls) who underwent kidney biopsy in our department because of abnormal urinary findings. These children were diagnosed histopathologically with IgAN between 2007 and 2014 at the Kindai University Hospital, Faculty of Medicine. During the observation period, kidney biopsies were performed at least twice: first, to make a definitive diagnosis and then to confirm the subsequent treatment response. Based on the first kidney specimen and clinical features, the prognosis was considered relatively or moderately unfavorable according to the clinical guidelines of the Japanese Society of Nephrology. All subjects received steroids and additional agents including an immunosuppressants, a renin-angiotensin-aldosterone (RAA) inhibitors, or angiotensin receptor antagonists (ARA), and warfarin (cocktail therapy); at the time of the second biopsy, all subjects had continuously received an RAA inhibitors or ARAs. Hypertension was defined as systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) that placed the patients in the 95th percentile considering gender, age, and height on at least 3 occasions.

Clinical evaluation items included height (cm), body weight (kg), creatinine clearance (Ccr, mL/min/1.73 m²), and the estimated glomerular filtration rate (eGFR, mL/min/1.73 m²). The eGFR was calculated using Schwartz' formula: \[ \text{eGFR} = \frac{k \times \text{height (cm)}}{\text{serum creatinine (mg/dL, according to enzymatic assay), where}} \]

Where \( k = 0.55 \) for boys and girls aged 2 to 12 years old; \( k = 0.70 \) for boys 13 aged or older; and 0.55 for girls aged 13 or older. To confirm differences in each of the above parameters between the periods of kidney biopsies were performed, the rate of change was calculated by the following formula: \[ \Delta \text{eGFR (\% per year)} = \left( \frac{\text{eGFR at the time of second biopsy} - \text{(eGFR at the time of first biopsy)}}{\text{interval (years between the two biopsies)}} \right) \times 100 \] Using a similar method, \( \Delta \text{BMI} \) was calculated.

Informed consent
This study was performed following approval by the Ethics Committee of Kindai University Faculty of Medicine and the acquisition of written informed consent from patients or their parents (approval number: 27-022).
Pathologic evaluation

Kidney specimens were obtained from all subjects by percutaneous biopsy, fixed in 10% formalin, embedded in paraffin, and cut into 3- to 4-μm sections for hematoxylin and eosin (HE), periodic acid-Schiff (PAS), periodic acid-methenamine silver (PAM), and Masson trichrome staining. Glomeruli in each specimen were counted, excluding glomeruli with focal sclerosis. The glomerular density (GD, /mm²) was calculated by dividing the number of glomeruli by total cortical area, measured using a computer-based image analyzer (NIS Elements; Nikon, Tokyo, Japan). The mean glomerular area (MGA, mm²) was determined. The glomerular volume (GV, μm³) was calculated with the formula: \( GV = (MGA)^{3/2} \times \beta / d \), where \( \beta \) is a dimensionless shape-specific coefficient (\( \beta = 1.38 \) for spheres), and \( d \) is a size distribution coefficient used to adjust for variations in glomerular size. To confirm differences in each histologic parameter, the rate of change was calculated using the following formula: \( AGD (\% \text{ per year}) = \frac{\text{GD at the second kidney biopsy} - \text{GD at the first biopsy}}{\text{GD at the first biopsy} \times \text{interval in years between the two biopsies}} \times 100 \). Using a similar method, \( AMGA \) was calculated. Sclerotic and crescent-forming glomeruli were counted in each specimen. The tubular interstitial lesions including tubular atrophy and interstitial fibrosis were scored from T0 to T4: T0 indicated absence of any lesions (normal); T1, minimal changes in some but not all sections; T2, slight changes that were present in all sections; T3 indicated moderate changes; and T4 indicated severe changes. Children whose specimen(s) included 10 or fewer glomeruli (including sclerotic glomeruli) were excluded from study.

Statistical analysis

Measurements are expressed as the mean ± standard deviation. Student’s t-test was used to compare results between first and second biopsy specimens. Parameters were compared based on individual Pearson correlation coefficients. A p value less than 0.05 was considered significant.

Results

Clinical and laboratory findings

Clinical and laboratory findings at the time of each biopsy are presented in Table 1. The number of patients was 22 (16 boys, 6 girls). Birth weights could be confirmed for 18 children (82%), whose mean birth weight was 2993 g (range, 1796 to 3742 g). Two LBW children (2000 g or less) were included (1796 and 1900 g, respectively). The median age at initial consultation was 10 years, 1 month (range, 4 years, 1 month to 14 years, 11 months). That at the first biopsy was 10.1 ± 2.5 years (range, 4.1 to 14.1).
The median age at the second biopsy was 12.8 ± 2.9 years (range, 5.1 to 17.3). The mean interval between the 2 biopsies was 2.0 ± 1.2 years (range, 1 to 6.5). No significant differences in BMI, systolic and diastolic blood pressures, serum creatinine, Ccr, or eGFR were evident between times of the first and second biopsies. Urinary protein, serum IgA, serum complement C3, and total cholesterol were significantly lower at the time of the second biopsy compared to the time of the first (Table 1).

**Histologic findings**

Respective total cortical areas of the first and the second renal biopsy specimens were 3.3 ± 0.98 × 10^6 mm² (range, 1.4 to 5.6 × 10^6) and 2.7 ± 0.93 × 10^6 mm² (range, 1.1 to 4.7 × 10^6). Numbers of glomeruli per unit area (glomerular density, or GD) of the first and the second specimens were 6.9 ± 2.2/mm² (range, 2.2 to 9.9) and 7.0 ± 2.4/mm² (range, 2.8 to 11.7) respectively. In the second specimen, GD had decreased in 11 of the 22 children (50%). However, overall GD differences between the first and second specimens were not significant. Mean glomerular areas of the first and the second specimens respectively were 7.6 ± 1.6 × 10^3 mm² (range, 4.9 to 10.8 × 10^3) and 8.1 ± 1.6 × 10^3 mm² (range, 5.8 to 11.5 × 10^3). No significant difference in MGA was found between the 2 specimens (Table 2). In 16 children (72.7%) with cellular crescent formation in the first specimen, no crescents were seen in the second specimen. In the first specimen, sclerotic glomeruli were observed in 12 children (54.5%); in 9 of these children, sclerotic glomeruli were fewer or absent in the second specimen. In 2 children without sclerotic glomeruli in the first specimen, sclerotic glomeruli were observed in the second specimen. The grade of tubular interstitial lesions was evaluated as T1 or lower, except in 1 child. No significant changes were in number (%) of sclerotic glomeruli, or in grade of fibrosis were noted between the first and the second time points.

**Association of glomerular density (GD) and area with other factors**

Negative correlations were seen between GD and MGA/maximum glomerular areas (MaxGA) in the second specimen in comparison with densities and areas in the first specimen. When GD was low, MGA and maxGA were increased (MGA, r = -0.645, P = 0.001; MaxGA, r = -0.508, P = 0.016; Figure 1). A negative correlation between GD and GV was noted in the second specimen (r = -0.677, P < 0.001; Figure 2). A negative correlation was seen between rate of change in GD (ΔGD) and GD in the first specimen (r = -0.473, P = 0.026; Figure 3), suggesting that even when GD was within the normal range in the first specimen, it could decrease later. When GD was low at the first time point, eGFR was high, representing a negative correlation (r = -0.424, P = 0.049; Figure 4). At the second time point, no significant relationship was evident. For comparison, adults with IgAN show a positive correlation between GD and the rate of change in Ccr.

**Table 2** Changes in histologic findings between the first and second renal biopsy specimens

<table>
<thead>
<tr>
<th></th>
<th>1st biopsy</th>
<th>2nd biopsy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD</td>
<td>range</td>
<td>mean±SD</td>
</tr>
<tr>
<td>Area of cortex (x10^6 mm²)</td>
<td>3.37±0.98</td>
<td>1.38 to 5.57</td>
<td>2.52±0.93</td>
</tr>
<tr>
<td>Number of glomeruli</td>
<td>20.0±8.7</td>
<td>8 to 42</td>
<td>18±7.87</td>
</tr>
<tr>
<td>Glomerular density (GD) (1/mm²)</td>
<td>7.2±2.2</td>
<td>2.12 to 9.93</td>
<td>7.3±2.39</td>
</tr>
<tr>
<td>Mean glomerular area (MGA) (x10^3 mm²)</td>
<td>7.7±1.6</td>
<td>5.0 to 10.8</td>
<td>7.7±1.6</td>
</tr>
<tr>
<td>Maximum glomerular area (MaxGA) (x10^3 mm²)</td>
<td>12.2±2.6</td>
<td>9.9 to 20.3</td>
<td>12.2±2.6</td>
</tr>
<tr>
<td>Glomerular volume (GV) (x10^6 µm³)</td>
<td>0.89±0.29</td>
<td>0.48 to 1.54</td>
<td>0.89±0.31</td>
</tr>
<tr>
<td>Maximum glomerular diameter (mm)</td>
<td>121.1±15.0</td>
<td>83.0 to 163.6</td>
<td>130.9±14.0</td>
</tr>
<tr>
<td>Maximum Bowman capsule diameter (mm)</td>
<td>138.1±17.7</td>
<td>96.7 to 184.2</td>
<td>139.9±15.9</td>
</tr>
<tr>
<td>Sclerotic glomeruli (%)</td>
<td>3.2±3.18</td>
<td>0 to 9.5</td>
<td>0±4.66</td>
</tr>
<tr>
<td>Crescent formation (%)</td>
<td>14.1±12.6</td>
<td>0 to 39.4</td>
<td>0</td>
</tr>
</tbody>
</table>
Changes of Glomerular Density in Childhood IgA Nephropathy

1. Positive correlations were evident between the systolic blood pressure (SBP) and MaxGA at the first time point (Figure 5), while no correlation was found between SBP and GD. At the second time point, no correlation was evident between GA and SBP. No correlation was seen between the diastolic blood pressure and glomerular area at either time point.

**Association between BMI and other factors**

When associations between BMI and other factors were examined at the first and second time points, BMI showed no correlation with any histologic factors such as GD, MGA, or GV (data not shown). Furthermore, we investigated the association of BMI at the first time point with a subsequent decrease in GD that is, the rates of change in GD or AGD. BMI showed no correlation with AGD ($r = -0.066, P = 0.770$). In addition, no correlations were seen between rate of change in BMI ($\Delta$BMI) and rates of change in eGFR ($\Delta$eGFR) and GD ($\Delta$GD).

**Association between birth weight and other factors**

Although no significant difference was evident, children with lower birth weights tended to be lighter ($r = 0.448, P = 0.072$; Figure 6a). A negative correlation was noted between the birth weight and total serum cholesterol at the first time point ($r = -0.531, P = 0.023$; Figure 6b); hypercholesterolemia was noted especially in
low-birth-weight children. On the other hand, no correlation was observed between birth weight and GD (data not shown).

**Discussion**

One study has suggested that glomerular enlargement might be a prognostic factor in children with IgAN. Searches obtained no similar assessments performed in children. The present retrospective study of 22 children with IgAN involved histologic evaluation of renal biopsy specimens in terms of GD at initial consultation and 2 years after the start of treatment, seeking associations between pathologic and clinical data. Overall treatment responsiveness was favorable. Improvements in clinical findings as well as decreases in proteinuria, serum IgA, and serum complement C3 were significant. As for histologic findings, the number of sclerotic glomeruli in the second specimen increased in 4 of the 22 children. In 3 of these children, chronic lesions in the first specimen were more advanced than in the others; although the grade

---

**Fig. 4** The relationship between GD and eGFR at the first time point and the second time point. When GD was low at the first time point, GFR is tended to be high, representing a negative correlation (P=0.049).

**Fig. 5** Positive correlation was found between SBP and MaxGA at the first time point. Correlations were not found between either SBP or MGA/MaxGA at the second time point.

**Fig. 6** (a) Children with lower birth weights tended to remain less heavy (P=0.072). (b) A negative correlation was noted between birth weight and total serum cholesterol at the first time point (P=0.023).
of mesangial proliferation was low. This advanced state may reflect a long interval between initial clinical abnormalities and the first biopsy, although these children showed low urinary protein excretion.

In 50% of the children GD had decreased in the second specimen while increasing slightly in the others, but overall change in GD between time points was not significant. This lack of conclusiveness may reflect the limitations of morphologic quantitation using tissue specimens obtained by a needle biopsy. Another possible explanation might involve differences in quantity and/or site of the specimen obtained. In general, fewer children had with histologic findings such as severely sclerotic glomeruli and tubulointerstitial disorder than reported in studies involving adults. In the present study, tissue damage seemed less likely to result in reduction in GD. Sclerotic glomeruli observed in the first specimen were fewer or absent in the second biopsy; possibly, some of these glomeruli may have been absorbed. In adults, high values for MaxGA and maximal glomerular diameter were reported to predict poor long-term prognosis. In the present study, negative correlations were seen between GD and MGA/MaxGA at the second time point, when negative correlations also were present between GD and GA/GV. Hypertension, obesity, diabetes, focal glomerulosclerosis, and polycystic kidney disease, which can excessively burden residual glomeruli, were not present in these children. Enlargement of glomeruli at the second time point might involve physiologic growth of individual glomeruli in addition to a response to low glomerular density.

At the first time point, a negative correlation was seen between GD and eGFR, but no such relationship was noted at the second time point. This result suggests that capillary stenosis and impingement upon the glomerular tufts by excessive mesangial matrix in the acute phase of IgAN directly decrease eGFR when GD is normal and adversely affect distribution of renal blood flow as well as eGFR when GD is reduced. One previous study indicated that a decrease in GD reduced kidney function in adults with IgAN, but no such tendency was evident in the present pediatric study. The mean interval between biopsies was 2.4 years, shorter than in the studies of adults. In the children, RAA inhibitors were administered continuously until the second biopsy.

In the second specimen fewer tissue abnormalities were noted, and glomerular tufts were large. In addition, all children received RAA inhibitors even after recovery. This may have contributed to the increase in eGFR. Furthermore, treatment-related tissue improvement was marked in these children, and advanced obesity was not present in our subjects. Although glomerular filtration volume increased in residual glomeruli because of decreased GD, whether filtration volume increases for the whole kidney is uncertain. At the first time point, positive correlations were evident between SBP and MaxGA. Actually neither MGA nor MaxGA was high in 4 children whose SBP was in the 95th percentile or higher, although hypertension is one cause of glomerular enlargement. Accordingly, this correlation may hold little significance. At the second time point, no relationship was noted between SBP and GA. Continuous administration of RAA inhibitors to all children may have been involved in this change.

BMI values were normal in most children in this study. Although no association was evident between BMI and clinical or histologic findings, the number of children in the 95th percentile for BMI increased from 1 to 4 between the 2 time points. In adults, BMI of 25 kg/m² or more was reported to predict poor long-term outcome, and high BMI was linked to decreasing GD. Considering a recent increase in prevalence of excess weight and obesity even in children, being overweight or obese may be considered an increasingly important risk factors for adverse outcome in children with glomerular disease including IgAN.

Significant correlations link the glomeruli number and size with LBW, another factor influencing kidney diseases. Only 2 of the present subjects had a history of LBW, no correlation between birth weight and GD was evident in this study. On the other hand, a negative correlation was seen between birth weight and serum total cholesterol at the first time point. Concerns about metabolic syndrome, which includes hypercholesterolemia, sometimes arise in early childhood, both in very-low-weight infants and in term infants who are small but of appropriate weight for gestational age. Unfortunately, the present study lack, detailed measurements such as cholesterol fractionation, triglyceride determinations, and lipoprotein fractionation.
as hypertension, obesity, and hypercholesterolemia may induce secondary glomerular damage. The onset of IgAN in such children with such problems may have increased risk of tissue abnormalities or treatment resistance. No significant data linking LBW, high BMI, or hypertension to an adverse clinical course in IgAN emerged from this study. However, since incidences of low birth weight, childhood obesity, hypertension, and dyslipidemia have increased recently and may contribute to increase in the future, long-term follow-up after clinical remission has been achieved may be necessary in children with these characteristics. Number of glomeruli does not increase during growth after birth, but rather decreases in the presence of various factors. This study suggested that even when GD in the first specimen is within the normal range, a decrease may occur later. Low GD is a risk factor for progression of CKD and for development of several renal disease. Glomerular disorders related to kidney diseases including IgAN may decrease the number of glomeruli. In particular, when tissue damage is marked, the number of intact glomeruli decreases even after clinical remission has been achieved. When consequent overloading of residual functional glomeruli occurs, nonimmunologic mechanisms of glomerular injury are thought to be responsible for ongoing decline of function. Continuous administration of RAA inhibitors may be necessary to prevent secondary glomerular derangement for children in whom overloading of residual glomeruli is suspected.

This study has several limitations. Little is known about whether GD in the renal biopsy specimens truly represented nephronal numbers in the whole kidney, since imaging data for total cortical volume in these patients were not available. Quantity of a biopsy specimen is restricted, especially children. Furthermore, the observation period may have been relatively short compared to some previous reports.

In conclusion, no significant differences were evident concerning glomerular density and apparent relationships to clinical prognosis compared with studies in adults. However, glomerular enlargement was evident in specimens with low glomerular density. Since the number of glomeruli tends to decrease with any kidney disease occurring during childhood that induces glomerular enlargement may affect prognosis in children with IgAN.

Authors' contributions

This study was authored by a special student of the Kindai University Graduate School, Faculty of Medicine. This manuscript is not under consideration for publication elsewhere, in any language, except as an abstract.

Acknowledgment

I am grateful to Dr. Tsukasa Takeamura (Department of Pediatrics, Kindai University Faculty of Medicine, Osaka, Japan) for technical assistance and useful comments.

Conflict of Interest and Consent Issues

I have no conflicts of interest affecting the present study. The use of the renal specimens for evaluation of the renal histologic conditions was approved by the patients and/or their guardians.

References

Changes of Glomerular Density in Childhood IgA Nephropathy