Clinicopathologic and Prognostic Study of Primary IgA Nephropathy With Light Chain λ Restriction in the Mesangial Deposits

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Introduction: Primary IgA nephropathy (IgAN) with light chain λ restriction in the mesangial deposits (IgAN-λ) has unique immunofluorescence (IF) features. Nevertheless, its clinicopathology and prognosis are still ambiguous.

Methods: From January 2002 to December 2020, the clinical and pathologic data of 3872 patients who were diagnosed with having primary IgAN by renal biopsy in our hospital were reviewed. A total of 187 patients who met the selection criteria for IgAN-λ were enrolled to conduct a retrospective single-center study. The selection criteria were that IF features conform to light chain λ restriction in the mesangial deposits. According to age, sex, renal function (estimated glomerular filtration rate [eGFR]), and follow-up time, the control group was constructed with 1:3 matched cases of IgAN. The clinicopathologic and prognostic differences between the 2 groups were analyzed.

Results: Compared with that in the IgAN group, the serum fibrinogen level in the IgAN-λ group was significantly higher (P < 0.001). Furthermore, cluster analysis indicated the different clusters involved in fibrinogen between the IgAN-λ and IgAN groups and that fibrinogen is associated with factors reflecting renal function in IgAN-λ but proteinuria levels in IgAN. The light chain λ deposit in the mesangium is associated with the formation of crescents in those with IgAN-λ, but complement C3 deposition in those with IgAN. Our Kaplan-Meier analysis revealed that the prognosis of the IgAN-λ group was significantly worse than that of the IgAN group within >6 years of follow-up (P = 0.02). The multi-Cox analysis revealed that the light chain λ restriction in the mesangial deposits was an independent risk factor for poor outcomes (eGFR decreased from the baseline ≥ 30% continuously or reached end-stage renal disease [ESRD] or died).

Conclusion: The prognosis of those with IgAN-λ was worse than that of those with IgAN, which may be attributed to the light chain λ restriction in the mesangial deposits inducing a significant systemic inflammation manifested as severe clinical features and frequent crescent.

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Lai et al. in 1986 reported that some IF results of primary IgAN revealed light chain λ restriction in the mesangial deposits (result of light chain κ staining was negative), which was different from common light chain co-dominance. Follow-up studies mainly focus on its mechanism. Suen et al. and Lai et al. speculated that the negative-charged light chain λ combined with the positive-charged unknown antigen in the kidney tissue led to selective deposition. Later, Lai et al. and Orfila et al. proposed that unknown specific antigens changed the produced light chain ratio κ/λ, resulting in excessive light chain λ. Vitro cell experiments revealed a noticeable difference in the light chain ratio κ/λ of IgA produced by stimulated and unstimulated cultured B lymphocytes, which provides strong evidence for this hypothesis. The above-mentioned studies were limited to monoclonal IgA deposition in the mesangium. Nevertheless, IgAN-λ was often observed when accompanied by other immunoglobulin deposition. As far as we have known, there are few detailed clinical studies involving light chain λ restriction in the mesangial deposits in this...
situation. Therefore, the clinicopathologic and prognostic characteristics of IgAN-λ remain to be clarified.

This study aimed to find out the clinical significance and value of light chain λ restriction in the mesangial deposits in IgAN, but it is not required to meet the criteria of monoclonal IgA deposition. A retrospective single-center research was conducted to compare the difference between the IgAN-λ group and the IgAN group in clinicopathology and prognosis, thus providing a reference for clinical diagnosis and treatment.

**METHODS**

**Patient Profiles**

From January 2002 to December 2020, the clinical and pathologic data of 3872 patients who were diagnosed with having IgAN by renal biopsy in our hospital were reviewed. A total of 187 patients who met the selection criteria for IgAN-λ were enrolled. The selection criteria for IgAN-λ were as follows: (i) IgAN was diagnosed by renal biopsy in our hospital and (ii) IF results confirmed light chain λ restriction in the mesangial deposits (the light chain λ staining was equal to or more than + and light chain κ staining was negative). The following patients were excluded: (i) accompanied with other primary or secondary glomerular diseases; (ii) IF results revealed that light chain κ staining was positive; (iii) immunofixation electrophoresis or bone marrow puncture indicated abnormality; (iv) clinical data were incomplete; and (v) pathologic diagnosis in our hospital was absent. The clinical and pathologic data of 187 patients who met the above-mentioned criteria were collected. In addition, according to age, sex, renal function (eGFR), and follow-up time, the control group was constructed with 1:3 matched cases of IgAN, and the clinical, pathologic, and prognostic differences between the 2 groups were analyzed. This is a retrospective single-center study. All data were collected from the hospital information system of The First Affiliated Hospital of Wenzhou Medical University, and the Ethics Committee of The First Affiliated Hospital of Wenzhou Medical University approved the protocol for data collection and masking of patient identifiable information.

**Clinical Parameters and Laboratory Data**

Variables of sex, age, height (m), weight (kg), blood pressure (mm Hg), hemoglobin (g/l), serum creatinine (s-Cr, mg/dl), serum albumin (g/l), uric acid (mg/dl), blood urea, total cholesterol (mmol/l), triglyceride (mmol/l), high-density lipoprotein—cholesterol (mmol/l), low-density lipoprotein—cholesterol (mmol/l), serum fibrinogen (g/l), serum light chain λ (mg/l), serum light chain κ (mg/l), and 24-hour proteinuria (g/d) were collected at diagnosis or at the time of first renal biopsy and considered the baseline level. The 24-hour proteinuria level was categorized into 3 according to the tertiles at baseline (low: 0.04–0.92 g/d; medium: 0.93–2.11 g/d; high: 2.12–19.95 g/d). The serum fibrinogen level was categorized into 3 according to the tertiles at baseline (low: 1.07–3.17 g/l; medium: 3.18–4.64 g/l; high: 4.65–10.52 g/l). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation, as follows: male: eGFR = 144 × (s-Cr/0.9)−0.411 × (0.993)gsre (s-Cr ≤ 0.9 ml/dl), eGFR = 144 × (s-Cr/0.9)−1.209 × (0.993)gsre (s-Cr > 0.9 ml/dl); female: eGFR = 144 × (s-Cr/0.7)−0.329 × (0.993)gsre (s-Cr ≤ 0.7 ml/dl), eGFR = 144 × (s-Cr/0.7)−1.209 × (0.993)gsre (s-Cr > 0.7 ml/dl). The eGFR was translated into 5 stages of chronic kidney disease according to the Kidney Disease Outcomes Quality Initiative guidelines.

**Histopathologic Examination**

All the specimens obtained from renal biopsy were examined by light microscopy and IF. Some of the specimens were examined by electron microscopy (EM). For light microscopy, all the specimens were stained with hematoxylin-eosin, periodic acid–Schiff, periodic acid–methylene silver, and elastic–Masson trichrome. For IF, frozen sections were stained with immunofixation electrophoresis or bone marrow. The pathologic diagnosis was based on the Oxford pathologic classification (MESTC-score [mesangial hypercellularity/endocapillary hypercellularity/segmental sclerosis/renal tubular lesion/crescent]) criteria, including mesangial hypercellularity (M0/1), endocapillary hypercellularity (E0/1), segmental glomerulosclerosis (S0/S1), tubular atrophy and interstitial fibrosis (T0/1/2), and crescent (C0/1). On the basis of results of light microscopy, statistics were made on the deposition sites of immunoglobulin (IgG, IgA, IgM), complement (C3, C4), fibrinogen, and light chain (κ, λ). The staining intensity was classified into – (not visible in low-power microscopy and not or seems to be visible in high-power microscopy), + (seems to be visible in low-power microscopy and visible in high-power microscopy), ++ (visible in low-power microscopy and clearly visible in high-power microscopy), +++ (clearly visible in low-power microscopy and dazzling in high-power microscopy), and ++++ (dazzling in both low- and high-power microscopy). If the
intensity fluctuates no >1 level, the higher level was chosen to represent the intensity (e.g., + to ++ was scored as ++). If the intensity fluctuates to >1 level, the mean level was chosen to represent the intensity (e.g., + to +++ was scored as ++). The staining intensity of the light chain $\lambda$ deposit in the mesangium was categorized into 2 levels according to the median (low: +; high: ++ to ++++). On the basis of results of EM, statistics were made on mesangial hypercellularity, mesangial matrix hyperplasia, basement membrane thickening, foot process fusion, and sites of electron-dense deposition.

Outcomes
The composite end point was defined as (i) eGFR decreased from the baseline $\geq$30% continuously or (ii) reaching ESRD or (iii) death.

Statistical Analysis
The variables of age, sex, eGFR, and follow-up time were matched among the IgAN-$\lambda$ and the IgAN groups using the propensity score matching algorithm with a ratio of 1:3. The numerical data are presented as the means (SD) or medians [interquartile range], and differences between groups were evaluated using variance analysis or the Kruskal–Wallis rank test. The categorical data are presented as counts with percentages (%), and differences between groups were analyzed using Pearson’s $\chi^2$ test. Benjamini and Hochberg method was used for correction of multiple comparisons. The correlation coefficient (Pearson for linear data and Kendall for ranked data) was calculated to plot the heatmap. Then, hierarchical clustering was used to perform a network to display the clustering relationship. The adjusted standardized residual was calculated and used to plot the mosaic plot. A multivariable Cox regression model was constructed and optimized using forward-backward stepwise methods and Akaike information criterion. The Kaplan–Meier curve was used to display the end point. All reported $P$ values were 2-tailed, and $P < 0.05$ was considered statistically significant. R version 4.0.4 and R packages (such as pheatmap,$^{15}$ vcd,$^{14}$ and surveminer$^{16}$) were used to perform the analyses and plots.$^{17,18}$

RESULTS

Clinical Parameters
According to the inclusion and exclusion criteria mentioned previously, a total of 187 patients with IgAN-$\lambda$ were selected from patients who underwent renal biopsy in our hospital from January 2002 to December 2020. The average age of the patients at diagnosis was 39 years old. The maximum age was 78 years old, and the minimum age was 19 years old. The male-to-female ratio was 1:1.67. Table 1 reveals the differences between the IgAN-$\lambda$ group (right column) and the IgAN group (left column). After matching age, sex, eGFR, and follow-up time, there was no significant difference in demographic data between the 2 groups. The IgAN-$\lambda$ group had significantly more average 24-hour proteinuria ($P = 0.04$), higher average serum fibrinogen level ($P < 0.001$), lower average serum albumin level ($P < 0.001$), higher average uric acid level ($P = 0.04$), higher average total cholesterol level ($P < 0.001$), higher average serum low-density lipoprotein—cholesterol level ($P = 0.02$), lower average serum light chain $\lambda$ and $\kappa$ level ($P < 0.001$), and lower $\kappa/\lambda$ ($P = 0.01$). Patients with IgAN-$\lambda$ got more aggressive treatment, using more steroids ($P < 0.001$), more immunosuppressants ($P < 0.001$), and more angiotensin II receptor blockers ($P < 0.001$). When using eGFR decreased from the baseline $\geq$30% continuously or reached ESRD or died as the composite end point, a marginal higher proportion of composite end point was observed in the IgAN-$\lambda$ group against the IgAN group (21.4 vs. 15.5%, $P = 0.09$).

The correlation matrix (Figure 1a) and network diagram (Figure 1b) of the clinical parameters of the 2 groups reveal obvious differences in the cluster in which serum fibrinogen level was correlated with between the IgAN-$\lambda$ group and the IgAN group. In the IgAN-$\lambda$ group, the serum fibrinogen level was associated with uric acid level and blood urea level and was most correlated with s-Cr level (Figure 1b). In the IgAN group, the serum fibrinogen level was correlated with total cholesterol and low-density lipoprotein—cholesterol and was most correlated with 24-hour proteinuria level (Figure 1b). The mosaic plot (Figure 1c) reveals that in the IgAN-$\lambda$ group, higher serum fibrinogen level may be associated with worse renal function, and the difference was more evident than that in the IgAN group. In the IgAN group, higher serum fibrinogen levels may be associated with more 24-hour proteinuria, and the difference was more evident than that in the IgAN-$\lambda$ group.

Renal Pathologic Manifestations

**Light Microscopy**

The results are found in Table 2. In this study, the average number of the glomeruli in the renal biopsy samples was 20.21 and the average number of global sclerosis was 3.84. The IgAN-$\lambda$ group had a significantly higher proportion of mesangial hypercellularity ($P < 0.001$), a higher proportion of endocapillary hypercellularity ($P = 0.05$), a higher proportion of tubular atrophy and interstitial fibrosis ($P < 0.001$), and a higher proportion of crescent...
formation ($P < 0.001$). In the IgAN-λ group, the crescents appeared in 93 cases, mostly small crescents, including 56 cases of the cellular crescent, 38 cases of the fibrocellular crescent, and 38 cases of the fibrous crescent. The proportion of fibrocellular crescent is higher ($P = 0.02$). The proportion of capillary loop necrosis was higher ($P < 0.001$). Most patients had renal interstitial inflammation, revealing interstitial lymphocyte and plasma cell infiltration, renal tubular atrophy, and renal interstitial fibrosis. A total of 8 cases accompanied acute interstitial inflammation, including 7 cases of mild inflammation and 1 case of moderate inflammation. In addition, 177 cases accompanied chronic interstitial inflammation, including 103 cases of mild inflammation, 58 cases of moderate inflammation, and 16 cases of severe inflammation. Most patients had varying degrees of vascular lesions, including mild endarterium hyperplasia in 64 cases, moderate endarterium hyperplasia in 25 cases, severe endarterium hyperplasia in 11 cases, and a higher proportion of hyalinosis ($P = 0.05$).

Immunoﬂuorescence

The results are found in Table 3. The ﬂuorescent tissue contains an average of 4.42 glomeruli (2–15 glomeruli). IgA is the dominant or co-dominant immunoglobulin deposited in the glomeruli (intensity + to ++++++). The mode of deposition is coarse granular or massive in the glomerular mesangial area and/or capillary wall. In the IgAN-λ group, the deposition rate of IgA in the capillary wall was 4.3%, which was signiﬁcantly higher than the IgAN group ($P = 0.01$) with higher staining intensity ($P = 0.02$). Of the cases, 93% had C3 deposition in the mesangial area, of which the proportion is signiﬁcantly higher ($P = 0.04$), and 2.7% in the capillary wall. The deposition rates of IgG and IgM in the mesangial area and capillary wall were 7.5%, 1.0% and 39.6%, 2.7%, respectively, which were not signiﬁcantly different from those in the IgAN group.

| Table 1. Baseline comparison of IgAN group and IgAN-λ group |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item            | IgAN            | IgAN-λ          | $P$ value       | Adjusted $P$ value |
| n               | 561             | 187             |                 |                  |
| Male (%)        | 215 (38.3)      | 70 (37.4)       | 0.8             | 0.8              |
| Age, yr (SD)    | 38.99 (13.00)   | 38.53 (12.53)   | 0.7             | 0.7              |
| BMI, mean (SD)  | 23.13 (3.49)    | 23.09 (3.59)    | 0.9             | 1                |
| MBP, mm Hg, mean (SD) | 98.17 (13.28)  | 96.73 (12.33)   | 0.2             | 0.3              |
| Follow-up mo, mo, mean (SD) | 34.22 (30.83)  | 35.53 (29.01)   | 0.6             | 0.7              |
| Proteinuria, g/d, mean (SD) | 1.86 (2.07)    | 2.29 (2.38)     | 0.02            | 0.04             |
| Hematuria, n %  | 495 (88.2)      | 173 (92.5)      | 0.1             | 0.2              |
| Hemoglobin, g/l, mean (SD) | 125.15 (18.43) | 124.84 (20.21) | 0.8             | 0.9              |
| Fibrinogen, g/l, mean (SD) | 3.47 (1.10)   | 4.28 (1.34)     | <0.001          | <0.001           |
| Serum albumin, g/l, mean (SD) | 38.01 (7.52)  | 34.47 (5.96)    | <0.001          | <0.001           |
| UA, mg/dl, mean (SD) | 6.03 (1.64)    | 6.36 (1.71)     | 0.02            | 0.04             |
| BU, mean (SD)   | 5.89 (2.71)     | 6.16 (2.82)     | 0.2             | 0.4              |
| Serum creatinine, mg/dl, mean (SD) | 1.12 (1.02)   | 1.09 (0.70)     | 0.7             | 0.7              |
| eGFR, ml/min per 1.73 m², mean (SD) | 91.36 (32.03) | 91.19 (35.56)   | 0.9             | 1                |
| Total cholesterol, mmol/l, mean (SD) | 4.88 (1.40)   | 5.38 (1.68)     | <0.001          | <0.001           |
| Triglyceride, mmol/l, mean (SD) | 1.86 (1.21)    | 1.96 (1.44)     | 0.3             | 0.5              |
| HDL, mmol/l, mean (SD) | 1.12 (0.29)    | 1.17 (0.33)     | 0.08            | 0.1              |
| LDL, mmol/l, mean (SD) | 2.88 (1.04)    | 3.13 (1.27)     | 0.008           | 0.02             |
| Serum light chain k, mg/l, mean (SD) | 8.60 (2.92)    | 6.94 (2.99)     | <0.001          | <0.001           |
| Serum light chain λ, mg/l, mean (SD) | 4.84 (1.61)    | 3.84 (1.70)     | <0.001          | <0.001           |
| κ/λ, mean (SD)  | 1.89 (0.37)     | 1.80 (0.36)     | 0.004           | 0.01             |
| Nephrotic syndrome, n (%) | 31 (5.5)       | 25 (13.4)       | 0.001           | 0.002             |
| End point, n (%) | 87 (15.5)       | 40 (21.4)       | 0.08            | 0.09              |
| Steroid, n (%)  | 161 (28.7)      | 83 (44.4)       | <0.001          | <0.001           |
| ACEI, n (%)     | 93 (16.6)       | 26 (13.9)       | 0.5             | 0.6              |
| ARB, n (%)      | 286 (51.0)      | 150 (80.2)      | <0.001          | <0.001           |
| CTX, n (%)      | 12 (2.1)        | 7 (3.7)         | 0.3             | 0.5              |
| Other IM = 1, n (%) | 39 (7.0)       | 35 (18.7)       | <0.001          | <0.001           |
| Steroid use duration, mo, mean (SD) | 1.04 (2.53)    | 4.28 (10.65)    | <0.001          | <0.001           |
| IM use duration, mo, mean (SD) | 0.67 (3.39)    | 2.89 (10.61)    | <0.001          | <0.001           |

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BU, blood urea; CTX, cyclophosphamide; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IgAN-λ, primary IgA nephropathy with light chain λ restriction in the mesangial deposits; IgAN, primary IgA nephropathy; IM, immunosuppressant; LDL, low-density lipoprotein; MBP, mean blood pressure; UA, uric acid.

All data were acquired before the renal biopsy.
Figure 1. Cluster analysis for clinical factors between the groups. (a) The heatmaps display the correlation coefficient (Pearson’s correlation coefficient for linear data) of the clinical factors in IgAN-λ and IgAN. The color label under the heatmap reveals that the red shade represents a positive correlation whereas the blue shade represents a negative correlation. The related factors shared the same or similar colors. (b) The network diagram displays the clinical factors’ clustering relationship in IgAN-λ and IgAN. The nodes represent the factors, and the shapes of the nodes represent the groups (circle for IgAN-λ and square for IgAN). The nodes connected by a solid black line reveal a stronger correlation and construct a cluster whereas the nodes connected by the solid gray line are the same factors from the different groups (the same factors are assigned a tiny weight, and different factors are assigned the same larger weight). The clusters, including the factor “Fibrinogen” of the 2 groups, are chosen to make cluster analysis by using the heatmap. (c) The mosaic plots display the relationships between levels of fibrinogen and CKD score/proteinuria levels in the 2 groups. The size of boxes is proportional to the frequency of each item. The shadings are (continued)
All cases were negative for light chain \( \kappa \) staining (Supplementary Figure S1) and positive for light chain \( \lambda \) staining (intensity + to ++++). The proportion of light chain \( \lambda \) deposition in the capillary wall was 5.8%, which was significantly higher than that in the IgAN group \( (P < 0.001) \).

**Electron Microscopy**

The results are found in Supplementary S1. Of the 187 patients with IgAN-\( \lambda \), 94 were examined by EM. Ultrastructurally, the deposition rate of electronic dense in the mesangial area was 69.1%, which was lower than that in the IgAN group \( (P < 0.001) \). The deposition rate of electronic dense in the paramesangial area was 41.5% higher than that in the IgAN group. Furthermore, the deposition was 6.4% in the subepithelial and 3.2% in the subendothelial. Most patients had foot process fusion. There were 42.6% of the cases who had diffuse foot process fusion, which was higher than the IgAN group \( (P = 0.004) \), and 48.9% of the cases had segmental foot process fusion. Some patients had thickened basement membrane, including 8 cases with mild thickening and 2 cases with moderate thickening.

The correlation matrix (Supplementary Figure S2A) and network diagram (Supplementary Figure S2B) of the pathologic parameters of the 2 groups reveal obvious differences in the cluster in which light chain \( \lambda \) deposit in the mesangium was correlated with between the IgAN-\( \lambda \) and the IgAN groups. In the IgAN-\( \lambda \) group, the light chain \( \lambda \) deposit in the mesangium was correlated with chronic inflammation in the renal interstitium, T1, and capillary loop necrosis and most correlated with C1 (Supplementary Figure S2B). Nevertheless, in the IgAN group, the light chain \( \lambda \) deposit in the mesangium was correlated with the IgA deposit in the mesangium and most correlated with the C3 deposit in the mesangium (Supplementary Figure S2B). The mosaic plot revealed that a high level of light chain \( \lambda \) deposit in the mesangium was significantly associated with a higher C-score and C3 deposition, either in the IgAN-\( \lambda \) or the IgAN group. Furthermore, the IgAN-\( \lambda \) group has an increasingly high level of light chain \( \lambda \) deposit in the mesangium. It is associated with a significantly increased crescent formation and C3 deposition proportion (Supplementary Figure S2C).

### Table 2. Light microscopy result comparison of IgAN group and IgAN-\( \lambda \) group

| Item | IgAN | IgAN-\( \lambda \) | \( P \) value | Adjusted \( P \) value |
|------|------|-------------------|--------------|------------------------|
| Number of glomeruli, mean (SD) | 20.16 (11.20) | 20.39 (10.58) | 0.8 | 0.8 |
| Global sclerosis, mean (SD) | 3.70 (4.28) | 4.25 (4.12) | 0.1 | 0.2 |
| M1, n (%) | 147 (26.2) | 81 (43.3) | <0.001 | <0.001 |
| E1, n (%) | 155 (27.6) | 67 (35.8) | 0.04 | 0.05 |
| S1, n (%) | 338 (60.2) | 118 (63.1) | 0.5 | 0.7 |
| T-score, n (%) | — | — | <0.001 | <0.001 |
| T0 | 431 (76.8) | 113 (60.8) | — | — |
| T1 | 97 (17.3) | 49 (26.3) | — | — |
| T2 | 33 (5.9) | 24 (12.9) | — | — |
| C1, n (%) | 59 (10.5) | 93 (49.7) | <0.001 | <0.001 |
| Mesangial hypercellularity, n (%) | 490 (87.3) | 174 (93.0) | 0.02 | <0.001 |
| Mesangial matrix expansion, n (%) | 497 (88.6) | 178 (95.2) | 0.007 | 0.01 |
| Endocapillary hypercellularity, n (%) | 141 (25.1) | 66 (35.3) | 0.009 | 0.02 |
| Adhesion, n (%) | 372 (66.3) | 120 (64.2) | 0.7 | 0.7 |
| Cases with crescents, n (%) | — | — | — | — |
| Cellular crescent | 138 (24.6) | 56 (29.9) | 0.2 | 0.3 |
| Fibrocellular crescent | 108 (19.3) | 54 (28.9) | 0.008 | 0.02 |
| Fibrous crescent | 88 (15.7) | 38 (20.3) | 0.2 | 0.3 |
| Intestinal inflammation change, n (%) | — | — | — | — |
| Acute-mild inflammation | 7 (1.2) | 7 (3.7) | 0.06 | 0.1 |
| Acute-moderate inflammation | 8 (1.4) | 1 (0.5) | 0.6 | 0.7 |
| Acute-severe inflammation | 0 (0.0) | 0 (0.0) | — | — |
| Chronic-mild inflammation | 327 (58.3) | 103 (55.1) | 0.5 | 0.7 |
| Chronic-moderate inflammation | 99 (17.6) | 58 (31.0) | <0.001 | <0.001 |
| Chronic-severe inflammation | 51 (9.1) | 16 (8.6) | 0.9 | 1 |
| Vascuropathy, n (%) | — | — | — | — |
| Mild endarterum hyperplasia | 152 (27.1) | 64 (34.2) | 0.07 | 0.1 |
| Moderate endarterum hyperplasia | 37 (6.6) | 25 (13.4) | 0.006 | 0.02 |
| Severe endarterum hyperplasia | 15 (2.7) | 11 (5.8) | 0.07 | 0.1 |
| Hyalinosis | 186 (33.2) | 80 (42.8) | 0.02 | 0.05 |
| Glomerular capillary loop necrosis | 33(5.9) | 34(20.9) | <0.001 | <0.001 |

C1, crescents in at least 1 glomerulus; E1, endocapillary hypercellularity; IgAN-\( \lambda \), primary IgA nephropathy with light chain \( \lambda \) restriction in the mesangial deposits; IgAN, primary IgA nephropathy; M1, mesangial hypercellularity; S1, segmental glomerulosclerosis; T0, normal; T1, tubular atrophy and interstitial fibrosis.

### Prognosis and Risk Factors

The average follow-up time was 34.56 months (2–100 months). When eGFR decreased from the
Table 3. Immunofluorescence result comparison of IgAN group and IgAN-λ group

| Item                        | IgAN        | IgAN-λ       | P value     | Adjusted P value |
|-----------------------------|-------------|--------------|-------------|------------------|
| Deposit site, n (%)         |             |              |             |                  |
| IgA in capillary wall       | 7(13.5)     | 8(4.3)       | 0.008       | 0.01             |
| IgA in mesangium            | 951(100)    | 187(100)     | 0.4          | 0.67             |
| IgM in capillary wall       | 34(6.1)     | 5(2.7)       | 0.3          | 0.4              |
| IgM in mesangium            | 261(46.5)   | 74(39.6)     | 0.1          | 0.2              |
| IgG in capillary wall       | 5(0.9)      | 2(1)         | 0.7          | 0.7              |
| IgG in mesangium            | 51(9.1)     | 14(7.5)      | 0.3          | 0.4              |
| C3 in capillary wall        | 13(2.3)     | 5(2.7)       | 0.8          | 1                |
| C3 in mesangium             | 482(86.0)   | 174(93.0)    | 0.01         | 0.04             |
| C4 in mesangium             | 0(0.0)      | 3(1.6)       | 0.01         | 0.04             |
| Fibrinogen in capillary wall| 23(4.1)     | 1(0.5)       | 0.03         | 0.06             |
| Fibrinogen in mesangium     | 101(18.0)   | 21(11.2)     | 0.004        | 0.005            |
| C in capillary wall         | 4(0.7)      | 0(0.0)       | 0.6          | 0.7              |
| C in mesangium              | 367(65.4)   | 0(0.0)       | <0.001       | <0.001           |
| λ in capillary wall         | 2(0.4)      | 11(5.8)      | <0.001       | <0.001           |
| λ in mesangium              | 388(65.1)   | 187(100)     | <0.001       | <0.001           |
| Intensity score             | 1/2/3/4 (median) | 1/2/3/4 (median) | 0.01         | 0.02             |
| IgA in capillary wall       | 0/2/5/0 (3) | 0/4/2/2 (3.5)| 0.01         | 0.02             |
| IgA in mesangium            | 9/85/44/19 (3)| 1/3/1/14/48 (3)| 0.9          | 1                |
| IgM in capillary wall       | 23/10/1/0 (2)| 3/2/0/0 (1)  | 0.07         | 0.09             |
| IgM in mesangium            | 196/60/5/0 (1)| 38/14/20 (1)| 0.09         | 0.1              |
| IgG in capillary wall       | 4/1/0/1 (1) | 1/0/1/0 (1)  | 0.8          | 0.8              |
| IgG in mesangium            | 32/17/2/0 (1)| 5/8/1/0 (2)  | 0.6          | 0.8              |
| C3 in capillary wall        | 2/8/3/0 (2) | 1/2/0/2 (2)  | 0.8          | 0.9              |
| C3 in mesangium             | 82/204/194/2 (2)| 18/87/68/0 (2)| 0.04         | 0.05             |
| C4 in mesangium             | 0/0/0/0 (0) | 2/0/1/0 (1)  | 0.003        | 0.008            |
| Fibrinogen in capillary wall| 16/7/0/0 (1)| 0/1/0/0 (1)  | 0.02         | 0.05             |
| Fibrinogen in mesangium     | 52/42/34/1 (4)| 4/12/5/0 (2)| 0.07         | 0.09             |
| x in capillary wall         | 4/0/0/0 (1) | 0/0/0/0 (0)  | 0.2          | 0.4              |

C, crescent; IF, immunofluorescence; IgAN-λ, primary IgA nephropathy with light chain λ restriction in the mesangial deposits; IgAN, primary IgA nephropathy.

The intensity of IF was classified as −, +, ++, +++, and ++++. An intensity no less than + was defined as positive. If the intensity fluctuates >1 level, the intensity was chosen to represent the intensity (e.g., + to ++ was scored as ++). If the intensity fluctuates ≤1 level, the mean intensity was chosen to represent the intensity (e.g., ++ to +++ was scored as ++). The intensity score 1/2/3/4 represents −, +++, ++, and +++, respectively, and the count of each score was found in the table. The χ2 test was used for intergroup comparison of deposit sites. Wilcoxon ranked sum test was used for intergroup comparison of the intensity score.

baseline ≥30% continuously or ESRD was reached or death as the composite end point, the prognosis of the IgAN-λ group was worse than that of the IgAN group (P = 0.02) (Figure 2). Using eGFR decreased from the baseline ≥30% continuously or reaching ESRD or died as the composite end point, univariate Cox regression analysis was performed on the related factors of IgAN-λ (Supplementary S2). The results of multivariable Cox regression are found in Table 4. The increased serum albumin level was an independent protective factor (P < 0.001), and the increased mean blood pressure (P < 0.001), increased s-Cr level (P < 0.001), light chain λ restriction in the mesangial deposits (P = 0.04), higher staining intensity of light chain λ deposit in the mesangium (P = 0.04), renal tubular atrophy or interstitial fibrosis (P < 0.001), increased 24-hour proteinuria (P = 0.01), increased serum fibrinogen level (P = 0.02), and increased triglyceride level (P = 0.006) were independent risk factors.

The mosaic plot reveals that higher serum fibrinogen levels in both groups may be associated with a worse prognosis (Supplementary Figure S3). In the middle/high level of the fibrinogen group, the difference in the IgAN-λ group is more obvious than that in the IgAN group. Higher staining intensity of light chain λ deposit may be associated with worse prognosis in the IgAN-λ group (Supplementary Figure S3). In the high staining intensity of the light chain λ group, the difference in the IgAN group is more obvious than in the IgAN-λ group. For the IgAN-λ group, in the high-level fibrinogen group and low staining intensity of the light chain λ group, the prognosis of those with immunosuppressant + steroid therapy was poorer, and the differences were obvious (Supplementary Figure S3).

**DISCUSSION**

Our study revealed that the IgAN-λ group had significantly higher serum fibrinogen levels than the IgAN group. The cluster analysis found that the serum fibrinogen level correlated with different clusters in the 2 groups, suggesting the inflammation may have different meanings. In the IgAN-λ group, the serum fibrinogen level was correlated with renal function indicators, especially the s-Cr level. Higher serum fibrinogen levels were associated with more severe deterioration of renal function, indicating the inflammation destroyed the structure and function of the kidney more directly. In the IgAN group, however, serum fibrinogen level was correlated with the indicators related to nephrotic syndrome, especially 24-hour proteinuria. As far as we have known, nephrotic syndrome may be the early stage of renal failure, which suggests that the IgAN-λ group progressed faster than the IgAN group and got more severe inflammation. Lai et al. found through experiments that the monoclonal light chain λ of IgA could further activate leukocytes to undergo chemotaxis and aggregation after the initial binding of leukocytes. Aggregated leukocytes caused inflammatory damage. On one hand, it caused the increase of serum fibrinogen levels, and on the other hand, it participated in the damage of the glomerular interstitium, which may lead to renal failure, which is consistent with our results. Previous studies proposed that patients with IgAN-λ relatively rarely progress to chronic kidney disease, and the probability of acute disease was not significantly different from patients with IgAN, but our study suggested that the lesions were more severe, which may not support the view.
Increased s-Cr (mg/dl) 1.30 (1.18–1.42) <0.001
Increased proteinuria (g/d) 1.12 (1.03–1.23) 0.01
Increased s-β2-m (mg/l) 1.30 (1.18–1.44) <0.001
Increased Alb (g/l) 0.93 (0.91–0.97) <0.001
Increased TGF (mmol/l) 1.21 (1.05–1.38) 0.006
Increased fibrinogen (g/l) 1.06 (1.02–1.10) 0.02
Increased s-κ (mg/l) 0.71 (0.70–0.90) 0.1
Increased s-λ (mg/l) 1.35 (1.32–1.68) 0.09

Pathologic factors
Light chain λ restriction in the mesangial deposits 1.54 (1.03–2.31) 0.03
T1/T2 5.05 (3.24–7.89) <0.001
Light chain λ intensity 1.34 (1.32–1.36) 0.04

AIC, Akaike information criterion; Alb, serum albumin; eGFR, estimated glomerular filtration rate; HR, hazard ratio; MBP, mean blood pressure; s-Cr, serum creatinine; s-κ, serum light chain κ; s-λ, serum light chain λ; T1/T2, tubular atrophy and interstitial fibrosis; TG, triglyceride.

This is the result of multivariable Cox regression model. The composite end point is the decrease of eGFR to <30% of the baseline or end-stage renal disease or reaching the end-stage renal disease or died. Factors with statistical differences (P < 0.1) in univariable Cox regression (as found in Supplementary S3) or considered meaningful in the cluster analysis were selected to build a multivariable Cox regression model. Then, the AIC using the forward-backward stepwise was performed to select the optimal model with the lowest AIC value. All the data were acquired before the renal biopsy.

Meanwhile, higher staining intensity led to a higher proportion of crescent formation, indicating a poor prognosis. In the IgAN group, the light chain λ deposit in the mesangium was correlated with C3 and IgA deposit in the mesangium, suggesting the relation to classic immune-mediated inflammation. Setoguchi et al.20 reported a case of IgAN-λ whose EM results revealed that the electronic dense was mainly deposited in the mesangium and subendothelium and thought that there were no characteristic structures. Our study found that except for more severe mesangial hypercellularity and a more severe degree of foot process fusion, patients with IgAN-λ had a higher proportion of electron-dense deposits in the para-mesangium than those with IgAN. This may relate to the light chain λ restriction mechanism in the mesangial deposits, which needs further study.

Previous studies21–25 proposed no significant difference in prognosis between patients with IgAN-λ and patients with IgAN. Nevertheless, the small sample size and short follow-up time may affect the power of tests and the conclusion. Similarly, our χ² test results revealed a marginal difference in the proportion of composite end points between the IgAN-λ and the IgAN groups (21.4 vs. 15.5%, P = 0.09). Nevertheless, the Kaplan–Meier analysis displayed a significantly decreased survival rate free from composite end points in the IgAN-λ group (log-rank, P = 0.02), which strongly indicated a poor prognosis in the IgAN-λ group. Furthermore, we found that higher mean blood pressure, more 24-hour proteinuria, higher triglyceride level, higher serum fibrinogen level, and worse renal function at diagnosis resulted in a poorer prognosis. This suggested that patients with hypertension, hyperlipidemia, inflammation, and renal insufficiency should be treated more aggressively. The prognostic difference caused by increased serum fibrinogen level is more obvious in the IgAN-λ group, especially in the high-level fibrinogen group, which indicated further that the inflammation destruction may be a cascade. It
also suggested that timely suppression of the inflammatory response and reducing the destruction of the kidney structure may be the key to therapy. The prognostic difference caused by the increased staining intensity of light chain λ deposit in the mesangium is more obvious in the IgAN-λ group, suggesting the staining intensity could be used to evaluate the prognosis.

In the high-level fibrinogen group, the steroid + immunosuppressive therapy resulted in an obvious poorer prognosis, which is supposed to be related to the serious condition of these patients at the beginning of treatment, and immunosuppressive therapy may not slow down the progression of the kidneys among these patients. Other effective treatment needs to be further studied. For patients with low staining intensity of light chain λ, the prognosis of steroid + immunosuppressive was obviously poorer than other therapies, which was different from the high staining intensity of the light chain λ group. It suggested that aggressive treatment may be related to poor prognosis for patients with nonserious renal pathologic manifestations, which requires further prospective studies.

Previous studies thought that the clinical significance of light chain λ restriction in the mesangial deposits in IgAN was still controversial, but our present study found it of great value. The research aimed to initially explore the impact of light chain λ restriction in the mesangial deposits on the clinicopathology and prognosis of IgAN. Nevertheless, this research was a single-center retrospective study. Further large-sample and multicenter research are needed.

**CONCLUSION**

Compared with patients with IgAN, those with IgAN-λ had significantly higher serum fibrinogen levels which correlated with renal function. Pathologic manifestation included a higher proportion of crescent formation, which correlated with light chain λ deposit in the mesangium. The prognosis of IgAN-λ was significantly worse than that of IgAN.

**DISCLOSURE**

All the authors declared no competing interests.

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