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Prediction of Diagnosis of Immunoglobulin A Nephropathy Prior to Renal Biopsy and Correlation With Urinary Sediment Findings and Prognostic Grading

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Several clinical markers correlate well with the diagnosis and prognosis of IgA nephropathy (IgAN). In the present study, we re-evaluated the usefulness of these four clinical markers for prediction of the diagnosis of patients with IgAN through a comparison between many more patients with IgAN and those with other types of renal diseases. 364 patients with IgAN and 289 with other types of renal disease were examined. An analysis was performed prior to renal biopsy, using clinical markers including, serum IgA, serum IgA/C3 ratio, number of red blood cells in urinary sediments, and urinary protein. Patients with IgAN were divided into four groups according to histopathological findings. Presence of microscopic hematuria, persistent proteinuria, high serum IgA levels, and the serum IgA/C3 ratios are useful for prediction of diagnosis of IgAN and distinguishing it from other renal diseases. Blood pressure, urinary protein, serum uric acid, renal function, and urinary sediment findings may be useful for prediction of prognostic grading in patients with IgAN. J. Clin. Lab. Anal. 22:114–118, 2008.

Key words: IgA nephropathy; IgA/C3 ratio; urinary sediment; urinary cast

INTRODUCTION

Immunoglobulin A (IgA) nephropathy is one of the most common forms of chronic glomerulonephritis worldwide and the long-term prognosis is relatively poor. It is characterized clinically by hematuria and/or proteinuria, and it shows a highly variable clinical course on the whole (1). Although the diagnosis can not be established without renal biopsy, several clinical markers that correlate well with the diagnosis and prognosis of IgA nephropathy have been reported. Some investigators have discussed the possibility of predicting the diagnosis and prognosis of this disease prior to renal biopsy (2,3).

We have already reported the importance of four clinical markers in the diagnosis of patients with IgA nephropathy or in the differential diagnosis with other types of nephritis and nephropathy as follows: more than five blood cells in urinary sediments, persistent proteinuria (urinary protein of more than 0.3 g/day), serum IgA level of more than 315 mg/dL, and serum IgA/complement 3 (C3) ratio of more than 3.01. Patients with three or four clinical markers were easily diagnosed as having IgA nephropathy in our reports in the past (4,5). In the present study, we reevaluated the usefulness of these four clinical markers for prediction of the diagnosis of patients with IgA nephropathy through a comparison between many more patients with IgA nephropathy and those with other types of nephritis or nephropathy. Then we examined statistical differences or correlation in various clinical indexes that have been widely reported (6) among the prognostic groups of patients with IgA nephropathy. We also focused on the usefulness of urinalysis for prediction of the prognosis of patients with IgA nephropathy, which has rarely been mentioned.

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PATIENTS AND METHODS

Prediction of Diagnosis of IgA Nephropathy Prior to Renal Biopsy

We examined 364 patients with IgA nephropathy (IgA group) and 289 patients with other types of nephritis or nephropathy (non-IgA group) who all underwent renal biopsy in our hospital from 1980 to 2005 (Table 1). The analysis was performed to distinguish between these two groups using four clinical markers (serum IgA level, serum IgA/C3 ratio, number of red blood cells in urinary sediments, and urinary protein level) obtained from all patients before renal biopsy. Since the international reference preparation (International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)/CRM470) was standardized in Japan, data on serum IgA and C3 levels measured before 1997 were recalculated by the following calculation formulas : new IgA levels = 0.95 × old IgA levels +5.5 (normal range: 110–410 mg/dL), and new C3 levels = 1.32 × old C3 levels –5.5 (normal range: 69–128 mg/dL). Urinalysis was performed using routine tests in our university. Urinary sediment was screened using a urine particle flow cytometer and then checked by manual microscopic methods in almost all patients.

The Mann-Whitney U-test or Student's *t*-test was used for statistical comparisons between many data. A *P*-value <0.05 was regarded as significant. To evaluate the usefulness of the presence of all four clinical markers for predictive diagnosis of IgA nephropathy and distinguishing it from other types of nephropathy or nephritis, logistic regression analysis was performed.

TABLE 1. Diagnosis of patients

Diagnosis	n
IgA nephropathy	364
Non-IgA nephropathy	289
Membranous nephropathy (MN)	81
Mesangial proliferative glomerulonephritis without deposition of IgA (non-IgA PGN)	45
Minimal change nephrotic syndrome (MCNS)	34
Focal/segmental glomerular sclerosis (FGS)	27
Lupus nephritis (LN)	27
Membranoproliferative glomerulonephritis (MPGN)	10
Henoch-Schönlein purpura nephritis (HSPN)	10
Post-streptococcal acute glomerulonephritis (PSAGN)	9
Multiple myeloma (MM)	8
Tubulointerstitial nephritis (TIN)	8
Alport's syndrome	6
Rapidly progressive crescentic glomerulonephritis (RPGN)	6
Diabetic nephropathy (DMN)	6
Thin basement membrane disease (TBMD)	6
Hepatitis virus associated nephritis	3
Amyloid nephropathy	1
Nephrosclerosis	1
Cryoglobulinemia	1

Correlation With Histopathological Findings in Renal Biopsy Specimens

Histological classification was based the prognostic criteria in Japan (Table 2) (7). We examined differences in systolic and diastolic blood pressure, serum IgA level, serum IgA/C3 ratio, creatinine clearance (Ccr), serum uric acid, urinary protein, urinary beta 2 microglobulin, number of red blood cells, and total number of each type of urinary casts (hyaline casts, granular casts, red

TABLE 2. Prognostic criteria for IgA nephropathy on the basis of the light microscopic findings of renal biopsy specimens*

Good prognosis group (Grade I)	Dialysis will probably never be required; slight mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation, and adhesion to Bowman's capsule are absent. Prominent changes are not observed in the interstitium, renal tubuli, or blood vessels.
Relatively good prognosis group (Grade II)	The likelihood of dialysis is relatively low; slight mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule is observed in less than 10% of all biopsied glomeruli. Interstitial and vascular findings are same as for good prognosis group.
Relatively poor prognosis group (Grade III)	Dialysis is likely to be required within 5–20 years. Moderate, diffuse mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule is observed in 10–30% of all biopsied glomeruli. Cellular infiltration is slight in the interstitium, except around some sclerosed glomeruli. Tubular atrophy is slight, and mild vascular sclerosis is observed.
Poor prognosis group (Grade IV)	The possibility of dialysis within 5 years is high. Severe, diffuse mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule is observed in more than 30% of all biopsied glomeruli. When sites of sclerosis are totaled and converted to global sclerosis, the sclerosis rate is more than 50% of glomeruli. Some glomeruli also show compensatory hypertrophy. Interstitial cellular infiltration and tubular atrophy, as well as fibrosis, are observed. Hyperplasia or degeneration may be observed in some intrarenal arteriolar walls. Among these histological findings, the rate of glomerular sclerosis and interstitial fibrosis are important markers for the classification of IgA nephropathy.

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blood cell [RBC] casts, white blood cell [WBC] casts, fatty casts, and oval fat bodies) in the urinary sediments in each prognostic group. The degree of correlation between prognostic grading and each clinical parameter was evaluated (8). Ccr was calculated using the Cockcroft-Gault formula. The degree of hematuria was scored in five phases, as follows: 1 = 1–5 RBCs/high power field (HPF); 2 = 6–10 RBCs/HPF; 3 = 11–15 RBCs/HPF; 4 = 16–20 RBCs/HPF; and 5 = more than 21 RBCs/HPF. Presence of each type of urinary cast was scored as 0 or 1 according to whether or not it was observed in several urinalysis before renal biopsy, and the total number of urinary casts was calculated. For example, the total numbers of cast types was 3 when hyaline casts, granular casts, and fatty casts were recognized simultaneously in urinary sediment inspection.

The significant differences between these data were tested by Student's *t*-test or Mann-Whitney U-test. In addition, we calculated the coefficient of correlation of Spearman or Pearson and examined the correlations between each of the clinical parameters. $P<0.05$ was regarded as significant.

RESULTS

Prediction of Diagnosis of IgA Nephropathy Prior to Renal Biopsy

Results for four clinical markers and incidences in the IgA group and non-IgA group are shown in Table 3. Levels of urinary protein and serum C3 were significantly lower in the IgA group ($P<0.01$). On the other hand, levels of the serum IgA and serum IgA/C3 ratio were significantly higher in the IgA group ($P<0.01$).

TABLE 3. Four clinical markers and incidence in IgA nephropathy and non-IgA nephropathy groups

	IgA nephropathy (n = 364)	non-IgA nephropathy (n = 289)	*P*
Urinary protein (g/day)[a]	1.25±0.16	3.68±0.33	<0.01
Serum IgA level (mg/dL)[a]	349.73±5.64	255.72±6.64	<0.01
Serum C3 level (mg/dL)[a]	97.37±1.13	103.91±1.86	<0.01
Serum IgA/C3 ratio[a]	3.81±0.09	2.39±0.02	<0.01
Incidence	n (%)	n (%)	*P*
RBC > 5/HPF	335 (92)	141 (49)	<0.01
Proteinuria ≥0.3 g/day	251 (69)	234 (81)	<0.01
Serum IgA ≥315 mg/dL	225 (62)	81 (28)	<0.01
Serum IgA/C3 ratio ≥3.01	233 (64)	79 (27)	<0.01

[a]Values are mean±SE.

Incidences of more than five red blood cells in urinary sediments, serum IgA of more than 315 mg/dL, and serum IgA/C3 ratio of more than 3.01 in the IgA group were significantly higher than those in the non-IgA group ($P<0.01$).

There was a significant difference in the presence of four clinical markers between IgA nephropathy and non-IgA nephropathy patients (Table 4a and b). The predictive value of diagnosis of IgA nephropathy prior to renal biopsy was 75% using our criteria with the presence of three or four clinical markers, and logistic regression analysis showed almost same results. Diseases showing high false-positive rates were lupus nephritis (59%), Henoch-Schönlein nephritis (50%), and post-streptococcal acute glomerulonephritis (44%). Diseases with low false-positives were nephrosclerosis, cryoglobulinemia, amyloid nephropathy, multiple myeloma, Alport syndrome, focal/segmental glomerular sclerosis, thin basement membrane disease, mesangial proliferative glomerulonephritis without IgA deposition, and tubulointerstitial nephritis. Each disease had a positive rate of less than 15%.

Correlation Between Prognostic Grading and Clinical Parameters

A total of 292 patients with IgA nephropathy were divided into four prognosis groups (Grade I–IV). Results for clinical parameters in each prognostic group are shown in Table 5. There were significant differences between each group in age, systolic blood pressure, urinary protein, urinary beta 2 microglobulin, Ccr, and serum uric acid level. The mean serum IgA level and serum IgA/C3 ratio gradually increased as the prognostic grading worsened, but there was no significant difference.

The total numbers of each type of urinary cast (hyaline casts, granular casts, RBC casts, WBC casts, fatty casts, and oval fat bodies) in the urine sediments before renal biopsy differed significantly among each prognosis group, especially for granular casts, fatty casts, and oval fat bodies appearing in the urinary sediment simultaneously ($P<0.01$). There was a positive relationship between prognostic grading and the total numbers of each type of urinary cast in the urinary sediments ($r = 0.423$, $P<0.01$).

DISCUSSION

Although a definite diagnosis of IgA nephropathy is made by renal biopsy, it is an invasive examination and has some contraindications at the time of clinical diagnosis (9). A noninvasive examination is important for deciding prognosis and treatment prior to renal biopsy and is important for obtaining informed consent

TABLE 4. Correlation between four clinical markers and glomerular disease

a. Logistic likelihood ratio test and coefficients

	Degrees of freedom	χ^2	*P*	Logistic regression coefficient	95% CI
RBC >5/HPF	1	125.412	<0.0001	–2.438	0.054–0.142
Proteinuria ≥0.3 g/day	1	12.066	0.0005	0.790	1.397–3.480
Serum IgA ≥315 mg/dL	1	16.216	<0.0001	–0.946	0.245–0.616
Serum IgA/C3 ratio ≥3.01	1	15.291	<0.0001	–0.904	0.257–0.638

b. Predictive value of diagnosis of IgA nephropathy

	Predicted IgA nephropathy	Predicted non-IgA nephropathy	Diagnostic rate
Biopsied IgA nephropathy	280	84	76.92%
Biopsied non-IgA nephropathy	72	217	75.09%
Total			76.11%

TABLE 5. Prognostic gradings and clinical parameters

	Grade I	Grade II	Grade I+II	Grade III	Grade IV		*P*
n	26	52	78	128	86		
Age[a]	28.26±2.28	28.79±1.31	28.61±1.15	32.34±0.93	34.73±1.24	*P*<0.05 (I+II vs. III,IV)	
s-BP (mmHg)[a]	114.65±2.27	118.50±2.18	117.22±1.65	117.69±1.29	123.00±2.07		*P*<0.05 (III vs. IV)
d-BP (mmHg)[a]	69.58±2.20	68.25±1.57	68.69±1.27	69.07±0.99	72.22±1.43		
Urinary protein (mg/dL)[a]	40.96±10.55	56.25±11.29	51.15±8.31	122.85±11.18	217.72±24.63	*P*<0.01 (I+II vs. III)	*P*<0.01 (III vs. IV)
Urinary protein (g/day)[a]	0.22±0.07	0.53±0.16	0.42±0.11	1.22±0.28	1.90±0.21	*P*<0.05 (I+II vs. III)	P<0.05 (III vs. IV)
Urinary β2 MG (μg/L)[a]	81.92±10.39	117.77±15.91	105.82±11.28	137.35±19.87	260.38±57.09		*P*<0.05 (III vs. IV)
Creatinine clearance[a]	113.69±6.02	107.42±3.49	109.51±3.07	100.02±2.38	85.53±3.06	*P*<0.05 (I+II vs. III)	*P*<0.01 (III vs. IV)
Serum uric acid (mg/dL)[a]	5.19±0.24	5.20±0.24	5.20±0.18	5.68±0.13	6.15±0.21	*P*<0.05 (I+II vs. III)	*P*<0.05 (III vs. IV)
Serum IgA (mg/dL)[a]	353.73±20.68	333.84±12.31	340.47±10.69	351.95±10.37	341.39±10.03	ns	
Serum C3 (mg/dL)[a]	101.37±3.61	94.08±2.92	96.51±2.30	97.05±1.73	95.95±2.54	ns	
Serum IgA/C3 ratio[a]	3.55±0.20	3.69±0.17	3.64±0.13	3.72±0.13	3.92±0.25	ns	
Hematuria (score)[a]	4.08±0.31	4.01±0.19	4.09±0.16	4.30±0.11	4.22±0.14	ns	
Urinary casts (total numbers of each type of urinary cast)[a]	2.50±0.32	3.23±0.20		4.27±0.11	4.56±0.16	*P*<0.05 (I vs.II)	*P*<0.01 (II vs. III)
Hyaline casts (%)	65%	94%	84%	94%	94%	*P*<0.01 (I vs. II)	*P*<0.05 (I+II vs. III,IV)
Granular casts (%)	50%	54%	52%	76%	80%	*P*<0.01 (I+II vs. III,IV)	
RBC casts (%)	65%	69%	68%	86%	78%	*P*<0.05 (I+II vs. III,IV)	
WBC casts (%)	27%	37%	33%	61%	60%	*P*<0.05 (I+II vs. III,IV)	
Fatty casts (%)	15%	31%	26%	44%	68%	*P*<0.01 (I+II vs. III,IV)	
Oval fat bodies (%)	27%	38%	35%	69%	74%	*P*<0.01 (I+II vs. III,IV)	

[a]Values are mean±SE.
ns, not significant.

or deciding the treatment regimen. To distinguish IgA nephropathy from other types of nephropathy or nephritis including secondary renal disease before renal biopsy, our criteria of four clinical markers are useful for a predictive diagnosis of IgA nephropathy (5). After exclusion of secondary glomerular diseases based on the

medical history and examination of physical findings, our clinical criteria are reliable in predictive diagnosis of IgA nephropathy. In the present study, it was confirmed that blood pressure, proteinuria, serum uric acid, and Ccr are important factors for determination of the prognosis of IgA nephropathy, supporting past reports (10–13). Furthermore, frequency of various casts in urinary sediments and total numbers of each type of urinary cast should be highly convincing data for prediction of the prognosis in IgA nephropathy patients prior to renal biopsy.

In the clinical course of IgA nephropathy, it has long been recognized that tubulointerstitial damage reflects renal prognosis (14,15). First, higher numbers of each type of cast (so-called "telescoped sediment") showed a more severe prognosis statistically. Second, simultaneous appearance of granular casts, fatty casts, and oval fat bodies in urinary sediments showed a poor prognostic grading in patients with IgA nephropathy. These findings are based on the following knowledge: 1) all processes causing renal dysfunction, i.e., glomerular mesangial cell proliferation, sclerosis of the mesangial matrix, crescent formation, and glomerulosclerosis and necrosis of renal tubular epithelium, participate in generation of granular casts; 2) fatty casts reflect persistent heavy proteinuria; and 3) oval fat bodies are a result of fatty degeneration of renal tubular epithelial cells due to heavy proteinuria. Thus, renal tubular damage causes urinary casts (16). Vikse et al. (17) also indicated that the presence of urinary granular casts is an important factor in the prognosis of IgA nephropathy and mesangioproliferative glomerulonephritis (MPGN). They mentioned the importance of tubulointerstitial damage as a histopathological predictor of renal prognosis, and suggested that urinary granular casts might induce interstitial fibrosis in the future. Careful observation of urinary sediments as convincing markers of renal tubulointerstitial disorders is important.

The four groups of prognostic classifications in the Clinical Guidelines for IgA Nephropathy in Japan, proposed by the Subcommittee for IgA Nephropathy of the Special Study Group on Progressive Glomerular Disease, Ministry of Health, Labor and Welfare of Japan (7) include evaluation of interstitial and vascular findings as prognostic criteria, and place particular emphasis on interstitial fibrosis. It appears that our four clinical markers for the diagnosis of patients with IgA nephropathy are useful in differential diagnosis of other types of nephritis and nephropathy. In addition, total numbers of each type of urinary cast in urinary sediments may be useful in prediction of prognosis in patients with IgA nephropathy prior to renal biopsy. Simultaneous appearance of granular casts, fatty casts, and oval fat bodies in urinary sediments is an especially important sign of poor prognosis in IgA nephropathy.

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