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Significance of Serum IgA Levels and Serum IgA/C3 Ratio in Diagnostic Analysis of Patients With IgA Nephropathy

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Diagnostic analysis of clinical markers including serum IgA levels and serum IgA/C3 ratio in patients with IgA nephropathy is described. One hundred patients with IgA nephropathy (IgA nephropathy group) and 100 patients with other primary glomerular diseases (non-IgA nephropathy group) were examined. The analysis was performed to distinguish between these two groups using four clinical markers: 1) more than five red blood cells in urinary sediments, 2) persistent proteinuria (urinary protein of more than 0.3 g/day), 3) serum IgA levels of more than 315 mg/dl, and 4) a serum IgA/C3 ratio of more than 3.01. Patients with three or four clinical markers were easily diagnosed as having IgA nephropathy in this study. Furthermore, there was a significant difference in these clinical markers between the good prognosis and relatively good prognosis groups (Groups I and II) and the relatively poor prognosis and poor prognosis groups (Groups III and IV) of IgA nephropathy patients. It appears that the presence of microscopic hematuria and/or persistent proteinuria, high serum IgA levels, and the serum IgA/C3 ratio are useful for distinguishing IgA nephropathy from other primary renal diseases. It is postulated that these clinical markers are also useful for diagnosis of IgA nephropathy without renal biopsy. J. Clin. Lab. Anal. 17:73–76, 2003.

Key words: serum IgA/C3 ratio; diagnosis; IgA nephropathy

INTRODUCTION

IgA nephropathy is a common form of chronic proliferative glomerulonephritis throughout the world and is clinically characterized by microscopic hematuria and/or proteinuria. IgA may play an important role in pathogenesis and development of this disease (1). Several investigators have reported that the serum levels of IgA are significantly increased in patients with IgA nephropathy (2,3) and it has been suggested that elevated serum IgA levels are valuable in the diagnosis of IgA nephropathy (4). The joint committee of the special study group on progressive glomerular diseases of the Ministry of Health, Labor and Welfare of Japan and the Japanese Society of Nephrology reported serum IgA of more than 350 mg/dl in adults as one of the diagnostic criteria for IgA nephropathy (5). Before 1997, assay results of immunoglobulins and complements were reported using various reagent manufacturers' units, which were decided by their own standards. Thus, a serum IgA level of 350 mg/dl was not based on standardized evidence. In 1997, the international reference preparation, CRM470, produced by IFCC was introduced in Japan (6). All manufacturers in Japan produce standard immunoglobulins and complements based on the international reference preparation CRM470. The new criterion for IgA nephropathy obtained by nephelometric immune assay based on the international reference preparation CRM470 was 315 mg/dl (7). The serum IgA levels were internationally standardized and were based on evidence. Using this standard, we recently reported the importance of measurement of IgA, C3, and the serum IgA/C3 ratio prior to renal biopsy. The serum IgA/C3 ratio is a good marker for distinguishing IgA nephropathy from non-IgA nephropathy together with serum IgA levels (7). The objective of the present study was to determine if the presence of microscopic hematuria or protenuria, increase of serum IgA levels, and ratio of serum IgA to C3 (serum IgA/C3 ratio) are useful in the diagnosis of patients with IgA nephropathy prior to renal biopsy.

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MATERIALS AND METHODS

Serum and Urine Samples

Serum and urine samples from 100 patients with IgA nephropathy (IgA nephropathy group) and 100 patients with other primary renal diseases (non-IgA nephropathy group) were obtained from Juntendo University Hospital. All patients were randomly selected by a non-medical staff using clinical records of the patients in our division. All of these patients showed a non-nephrotic state and normal renal function tests (creatinine clearance of more than 70 ml/min, serum creatinine levels of less than 1.2 mg/dl), and were histologically diagnosed by renal biopsy. Patients with IgA nephropathy whose biopsy specimens stained predominantly for IgA in glomerular mesangial areas were included after exclusion of patients with systemic lupus erythematosus (SLE), Henoch-Schoenlein purpura (HSP) nephritis, liver cirrhosis, or other systemic diseases. IgA nephropathy patients were divided into four groups at the time of renal biopsy as follows (1). 1) Good prognosis group (Group I): there was almost no possibility of dialysis. Slight mesangial proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule were not observed. Prominent changes were not seen in the interstitium, renal tubuli or blood vessels. 2) Relatively good prognosis group (Group II): possibility of dialysis was relatively low. Slight mesangial proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule were observed in less than 10% of all biopsied glomeruli. Interstitial and vascular findings were the same as those in the good prognosis group. 3) Relatively poor prognosis group (Group III): dialysis was likely to be required within 5 to 20 years. Moderate, diffuse mesangial cell proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, and/or adhesion to Bowman's capsule were seen in 10% to 30% of all biopsied glomeruli. Cellular infiltration was slight in the interstitium except around some sclerosed glomeruli. Tubular atrophy was slight, and mild vascular sclerosis was observed. 4) Poor prognosis group (Group IV): the possibility of dialysis within 5 years was high. Severe, diffuse mesangial cell proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, and/or adhesion to Bowman's capsule were seen in more than 30% of all biopsied glomeruli. When sites of sclerosis were totaled and converted to global sclerosis, the rate of sclerosis was more than 50% of all glomeruli. Some glomeruli also showed compensatory hypertrophy. The rate of sclerosis was the most important index in the evaluation of prognosis. Interstitial cellular infiltration and tubular atrophy, as well as fibrosis were also observed. Hyperplasia or degeneration was present in some intrarenal arteriolar walls. Glomeruli often showed a mild-to-moderate increase in mesangial cells and matrices.

Thirty-five patients with diffuse or focal mesangial proliferative glomerulonephritis without mesangial IgA deposition (non-IgA PGN), 30 patients with membranous nephropathy (MN), 20 with minor glomerular abnormalities, 10 with focal glomerular sclerosis (FGS), 10 with thin basement membrane disease, and five patients with membranoproliferative glomerulonephritis (MPGN) were also selected.

None of the patients was treated with antiplatelet drugs, anti-inflammatory drugs, corticosteroids, and/or immunosuppressants at the time of renal biopsy. All serum and urine samples were obtained from the patients at the time of renal biopsy.

Detection of Serum IgA and C3 Levels

The levels of serum IgA and C3 were measured by the Hitachi (Tokyo, Japan) automated TIA A-NDII using an immunoturbidity method and the domestic standard in Japan (8), which was different from the new IFCC/CRM 470 in our hospital. In our hospital, these levels were recalculated to the new levels by the following formulas. New IgA levels $= 0.95 \times$ old IgA levels $+ 5.5$ (normal range: 110–410 mg/dl). New C3 levels $= 1.32 \times$ old C3 levels–5.5 (normal range: 69–128 mg/dl) (Juntendo Hospital Reports, unpublished). The new data recalculated by these formulas were significantly correlated with those obtained by immunoturbidity methods using an IFCC/CRM 470 (Auto TIA A-ND II, Nissui, Tokyo). The ratio of serum IgA to C3 (serum IgA/C3 ratio) was then calculated.

Evaluation of Clinical Markers and Statistical Analysis

Urinalysis was performed using the routine tests in our university. Presence of red blood cells in urinary sediments at more than five per high power field (HPF), persistent proteinuria of more than 0.3 g/day, serum IgA levels of more than 315 mg/dl, and serum IgA/C3 ratio of more than 3.01 were used for diagnostic analysis. Statistical analysis was performed using the chi-square test and stepwise regression analysis (forward selection method). $P < 0.05$ was regarded as significant.

RESULTS

Incidence of Four Clinical Markers in the IgA Nephropathy and Non-IgA Nephropathy Groups

The incidence of the four clinical markers is shown in Table 1. The incidences of more than five red blood cells

TABLE 1. Incidence of four clinical markers in IgA nephropathy and non-IgA nephropathy groups at the time of renal biopsy

Group	RBC more than 5/HPF	Persistent proteinuria	Serum IgA of more than 315 mg/dl	Serum IgA/C3 ratio of more than 3.01
IgA nephropathy (n = 100)	91[a]	77	73[a]	89[a]
Non-IgA nephropathy (n = 100)	45	74	36	45

[a] $P < 0.002$ vs. non-IgA nephropathy.
RBC, red blood cells in urinary sediments; HPF, high power field.

TABLE 2. Odds ratio for distinguishing IgA nephropathy from non-IgA nephropathy

	IgA	IgA/C3
Odds ratio	2.33	4.74
95% CI	1.08–5.03	1.96–11.49
P value	0.03	0.0006

TABLE 3. Correlation between the number of clinical markers and glomerular diseases

Group	Three or four clinical markers	One or two clinical markers
IgA nephropathy (n = 100)	82[a]	18
Non-IgA nephropathy (n = 100)	35	65

[a] $\chi^2 = 43.579$, $P < 0.001$ vs. non-IgA nephropathy.

in urinary sediments and persistent proteinuria of more than 0.3 g/day in IgA nephropathy were higher than those in non-IgA nephropathy, but there was no significant difference. The incidence of serum IgA of more than 315 mg/dl or serum IgA/C3 ratio of more than 3.01 in IgA nephropathy was significantly higher than that in the non-IgA nephropathy group ($P < 0.002$). The odds ratio for distinguishing IgA nephropathy from non-IgA nephropathy was significantly associated with levels of serum IgA and the ratio of serum IgA to C3 ($P = 0.03$, $P = 0.0006$, respectively) (Table 2). There was a significant difference in the presence of three or four clinical markers between IgA nephropathy and non-IgA nephropathy ($P < 0.001$) (Table 3). There was also a significant difference in the presence of all four clinical markers between IgA nephropathy and non-IgA nephropathy ($P < 0.0001$).

Relationship Between Prognostic Gradings and Four Clinical Markers in the IgA Nephropathy Groups

Prognostic gradings and four clinical markers in the IgA nephropathy groups are shown in Table 4. There was no significant difference in the incidence 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 among the four groups. However, the incidences of persistent proteinuria of more than 0.3 g/day in Groups III and IV, i.e., relatively poor to poor prognosis groups, were significantly higher than those in Groups I and II, i.e., good to relatively good prognosis group ($P < 0.0001$) (Table 5). There was no significant difference in the presence of more than three clinical markers between Groups III and IV, and Groups I and II.

DISCUSSION

IgA nephropathy is clinically characterized by microscopic hematuria and/or proteinuria. The indicative criteria for renal biopsy used in our division are as follows: presence of persistent proteinuria with or without microscopic hematuria, and almost normal range of renal function. Several researchers have previously reported that about half of the patients with IgA nephropathy showed high levels of serum IgA (2,3). The authors reported that serum IgA levels in patients with IgA nephropathy were significantly higher than those in patients with other glomerular diseases (7,8). Their results showed that the serum IgA/C3 ratio was also a useful marker for distinguishing IgA nephropathy from non-IgA nephropathy. These findings confirmed our previous data using IFCC/CRM 470. Although the gold standard for patients with IgA nephropathy is renal biopsy, general physicians find it difficult to perform biopsies. It appears that measurement of the ratio of serum IgA to C3 (serum IgA/C3 ratio) may be useful for diagnosis and prognostic grading in IgA nephropathy patients without renal biopsy (7).

In 1991, Yagame et al. (9) reported the significance of levels of circulating IgA-class immune complexes in discriminant analysis of patients with IgA nephropathy before renal biopsy. The correct classification rate was 80.0% using five clinical markers: serum IgA, microhematuria, IgA-circulating immune complexes (CIC), serum creatinine, and blood urea nitrogen. It was shown that the levels of serum IgA and IgA-CIC were major markers for clinical diagnosis of patients with IgA

TABLE 4. Prognostic gradings and four clinical markers in IgA nephropathy group

Group	RBC more than 5/HPF	Persistent proteinuria	Serum IgA of more than 315 mg/dl	Serum IgA/C3 ratio of more than 3.01
I (good prognosis) (n = 16)	16	4	12	15
II (relatively good prognosis) (n = 15)	14	11	9	15
III (Relatively poor prognosis (n = 42)	35	36	32	37
IV (poor prognosis) (n = 27)	26	26	20	22

RBC, red blood cells in urinary sediments; HPF, high power field.

TABLE 5. Incidence of four clinical markers in mild (group I/II) and advanced (group III/IV) IgA nephropathy groups

Grade	RBC more than 5/HPF	Persistent proteinuria	Serum IgA of more than 315 mg/dl	Serum IgA/C3 ratio of more than 3.01
I + II ($n = 31$)	30	15	21	30
III + IV ($n = 69$)	61	62[a]	52	59

[a] $P < 0.0001$ vs. I + II.
RBC, red blood cells in urinary sediments; HPF, high power field.

nephropathy using a discriminant analysis (9). In this study, we used four clinical markers, i.e., more than five red blood cells per HPF in urinary sediments, persistent proteinuria (urinary protein of more than 0.3 g/day), serum IgA levels of more than 315 mg/dl, and a serum IgA/C3 ratio of 3.01, to distinguish the IgA nephropathy group from the non-IgA nephropathy group according to our renal biopsy criteria and our previous data (7,8). Patients with three or four clinical markers were easily classified into the IgA nephropathy or non-IgA nephropathy groups in this study. The odds ratio for distinguishing IgA nephropathy from non-IgA nephropathy was also significantly associated with levels of serum IgA and ratio of serum IgA to C3 in using stepwise regression analysis. Furthermore, there was a significant difference in the incidence of clinical markers between the good prognosis and relatively good prognosis groups (Groups I and II) and the relatively poor prognosis and poor prognosis groups (Groups III and IV) of patients with IgA nephropathy. It appears that the presence of microscopic hematuria and/or persistent proteinuria, high serum IgA levels, and the serum IgA/C3 ratio are useful for distinguishing IgA nephropathy from other primary renal diseases. It is postulated that these clinical markers are also useful for diagnosis of IgA nephropathy without renal biopsy.

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