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Measurement of Serum IgA and C3 May Predict the Diagnosis of Patients With IgA Nephropathy Prior to Renal Biopsy

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The levels of serum IgA and C3 in patients with IgA nephropathy were determined using international standard serum (IFCC/CRM470) in a multicenter trial in Japan. The ratio of serum IgA to C3 (serum IgA/C3 ratio) without any information from renal biopsy was used for the diagnosis of IgA nephropathy. Three hundred and six patients with IgA nephropathy and other glomerular diseases, and 418 healthy adults were examined. The new diagnostic standardized criterion in patients with IgA nephropathy, obtained by nephelometric immune assay based on the international reference preparation CRM470, was 315 mg/dl. The serum IgA/C3 ratio was a more useful marker for distinguishing IgA nephropathy from non-IgA nephropathy together with serum IgA levels. This suggests that the measurement of serum IgA and C3 may predict the diagnosis of patients with IgA nephropathy prior to renal biopsy. J. Clin. Lab. Anal. 14:220–223, 2000.

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

INTRODUCTION

IgA nephropathy is a common form of chronic glomerulonephritis and is generally presumed to be an immune-complex-mediated glomerulonephritis (1). IgA may play an important role in pathogenesis and development of this disease. Several investigators reported that the serum levels of IgA are significantly increased in patients with IgA nephropathy (2,3). It has been suggested that elevated serum IgA levels are valuable in the diagnosis of IgA nephropathy (4). Therefore, it is important to quantitate precise amounts of serum IgA in patients with various types of chronic glomerulonephritis. The authors already reported that the levels of IgA in sera measured by laser nephelometry (LN) were significantly higher than those in sera measured by single radial immune diffusion (SRID) in patients with IgA nephropathy associated with increased levels of serum IgA (5). It appears that an increase of serum IgA in patients with IgA nephropathy may be due to an increase of polymers rather than of a monomer of IgA (5).

In 1995, the joint committee of the special study group on

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progressive glomerular diseases, the Ministry of Health and Welfare of Japan and the Japanese Society of Nephrology, reported that serum IgA of more than 350 mg/dl in adults is a frequent finding and a diagnostic criterion for IgA nephropathy (6). Although the levels of serum complement 3 (C3) tend to decrease in the active stage of chronic glomerulonephritis, it is generally considered that serum C3 levels show a normal range in patients with IgA nephropathy. However, there are differences in the levels of serum immunoglobulins and complement among institutions in Japan because of different methods used by each hospital. Recently, a new, widely accepted reference material (IFCC/CRM470) was standardized for measurement of IgG, IgA, IgM, C3, and C4 in sera (7). The objectives of the present study were: (1) to determine the levels of serum IgA and C3 in patients with IgA nephropathy using international standard serum (IFCC/CRM470) in a multicenter trial in Japan; and (2) to diagnose IgA nephropathy using the ratio of serum IgA to C3 (serum IgA/C3 ratio) without any information from renal biopsy.

MATERIALS AND METHODS

Serum Samples

Serum samples from 195 patients with IgA nephropathy and 111 patients with other glomerular diseases (non-IgA nephropathy) were obtained from our hospitals. Serum samples from 418 healthy adults (255 males, 163 females) were used as controls. All these patients were non-nephrotic. Patients with IgA nephropathy whose biopsy specimens stained predominantly for IgA in the glomerular mesangial areas were included after exclusion of patients with SLE, Henoch-Schoenlein purpura (HSP) nephritis, liver cirrhosis, or other systemic diseases. Among IgA nephropathy patients, 100 were males and 95 were females. The ages of the patients ranged from 22 to 71 years (mean: 37.9 years). Of the non-IgA nephropathy patients, 51 were male and 60 were female. The ages of these patients ranged from 12 to 79 years (mean: 42.3 years). Eighty-three patients with diffuse or focal mesangial proliferative glomerulonephritis without mesangial IgA deposition (non-IgA PGN), 21 patients with membranous nephropathy (MN), and 7 patients with membranoproliferative glomerulonephritis (MPGN) were used. None of the patients was treated with antiplatelet drugs, anti-inflammatory drugs, corticosteroids, and/or immunosuppressants at the time of renal biopsy.

All serum samples were obtained from the patients before renal biopsy. Serum samples were stored at –20°C or –70°C prior to use.

Detection of Serum IgA and C3

The levels of serum IgA and C3 were measured by the automated Dade Behring Nephelometer II(BNII) using a nephelometric immunoassay (8). In an immune-chemical reaction, immunoglobulins in human serum samples formed immune complexes with specific antibodies from a rabbit. These complexes scattered a beam of light passed through each sample. The intensity of the scattered light was proportional to the concentration of the relevant immunoglobulin in the samples. The results were evaluated by comparison with a standard known concentration.

The N-antiserum IgA and N-antiserum complement factor (C3c) produced by Dade Behring were used in this study. N-antiserum to human IgA or C3c was liquid animal serum and was produced by immunization of rabbits with highly purified IgA and C3c. The N-protein standard serum was used for preparing reference curves on the Dade Behring Nephelometer II. The N-protein standard serum consisted of pooled human sera. The assigned values were based on the IFCC reference material CRM470 (7). In order to distinguish IgA nephropathy and non-IgA nephropathy, the serum IgA/C3 ratio was calculated. Receiver-operating characteristic (ROC) plots were constructed to compare the diagnostic sensitivity or specificity of the serum IgA and the serum IgA/C3 ratio. The discriminant function for serum IgA and C3 was used to distinguish IgA nephropathy from non-IgA nephropathy as follows: Score = $0.023 \times C3 - 0.006 \times IgA - 0.791$.

Statistical Analysis

Statistical analysis was preformed using STAT FLEX (version 5.0) (9). The Student *t*-test was also used in statistical comparisons between individual study groups. $P < 0.05$ was regarded as significant.

RESULTS

Levels of Serum IgA and C3 in Patients With IgA Nephropathy, Non-IgA Nephropathy, and Healthy Adults

Results of serum IgA and C3 levels are summarized in Tables 1 and 2. The 95% range of serum IgA in patients with IgA nephropathy was from 146 to 650 mg/dl. The median and mean values of serum IgA in patients with IgA nephropathy were 315 mg/dl and 336 mg/dl, respectively. The mean values of serum IgA in patients with IgA nephropathy (336 ± 129 mg/dl, mean ± SD were significantly higher than those in patients with non-IgA nephropathy (270 ± 112 mg/dl) or healthy adults (230 ± 85 mg/dl) ($P < 0.001$). There were no significant differences in the levels of serum IgA between the patients with non-IgA nephropathy and those with healthy adults.

The 95% range of serum C3 in patients with IgA nephropathy was from 75 to 175 mg/dl. The mean value of serum C3 in patients with IgA nephropathy was 114 mg/dl, while that in patients with non-IgA nephropathy was 131 mg/dl. The levels of serum C3 in patients with IgA nephropathy were significantly lower than those in patients with non-IgA

TABLE 1. Levels of serum IgA (mg/dl) in patients with IgA nephropathy, non-IgA nephropathy, and healthy adults

	Healthy adults	IgA nephropathy	Non-IgA nephropathy
Number	418	195	111
Mean	230	336	270
S.D.	85	129	112
Median	215	315	250
Lower limit	110	146	112
Higher limit	410	650	540

nephropathy ($P < 0.001$). There was no significant difference in the levels of serum C3 between non-IgA nephropathy patients and healthy adults.

Comparison of IgA Nephropathy and Non-IgA PGN Using Diagnostic Specificity and Sensitivity

The diagnostic sensitivity or specificity, the discriminant efficiency among the levels of serum IgA or C3, the serum IgA/C3 ratio, and the discriminant function of serum IgA and C3 are summarized in Tables 3 and 4, and Figure 1. The mean values of the serum IgA/C3 ratio in patients with IgA nephropathy, non-IgA nephropathy, or healthy adults were 3.01, 2.21, or 1.89, respectively. The area under the serum IgA/C3 ratio curve was significantly larger than that under the serum IgA curve. The results from receiver-operating characteristic (ROC) plots showed that the diagnostic ability of the discriminant function was the same as the that of the serum IgA/C3 ratio (Fig. 1). The highest discriminant efficiency was 75 for the discriminant function of serum IgA and C3. The next highest discriminant efficiency was 73 for the serum IgA/C3 ratio. The discriminant efficiency of serum IgA was 67 (Table 4).

DISCUSSION

The joint committee of the special study group on progressive glomerular diseases, the Ministry of Health 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 (6). However, when they reported the diagnostic criteria, the assay results for immunoglobulins and complements were not standardized based

TABLE 2. Levels of serum C3 (mg/dl) in patients with IgA nephropathy, non-IgA nephropathy, and healthy adults

	Healthy adults	IgA nephropathy	Non-IgA nephropathy
Number	416	195	111
Mean	122	114	131
S.D.	22	24.6	46.7
Median	120	110	127
Lower limit	86	86	48
Higher limit	160	160	235

TABLE 3. Levels of serum IgA/C3 in patients with IgA nephropathy, non-IgA nephropathy, and healthy adults

	Healthy adults	IgA nephropathy	Non-IgA nephropathy
Number	418	195	111
Mean	1.89	3.01	2.21
S.D.	1.13	1.16	1.10

on international standards. The IgA level of 350 mg/dl was decided by the opinion of a professional society. Therefore, a serum IgA level of 350 mg/dl was a consensus value and was not based on standardized evidence. Before 1997, the assay results of immunoglobulins and complements were reported using different manufacturer's units, which were decided by their own standards. In 1997, the international reference preparation, CRM470, which was produced by IFCC, was introduced in Japan (7). All manufacturers in Japan had produced reagents for immunoglobulins and complements based on the international reference preparation CRM470. As a result, the differences between manufacturer's units for assay results of immunoglobulins and complements were decreased.

The new diagnostic criterion of the serum IgA levels in patients with IgA nephropathy was first established from this study. The criterion for IgA nephropathy obtained by nephelometric immune assay based on the international reference preparation CRM470 was 315 mg/dl. This serum IgA level is internationally standardized and also based on evidence. Therefore, it is necessary to change the diagnostic criterion from 350 mg/dl to 315 mg/dl officially in Japan. The serum IgA/C3 ratio and discriminant function for serum IgA and C3 were used for distinguishing IgA nephropathy from non-IgA nephropathy more efficiently because statistical evaluation by factor analysis showed that the levels of serum IgA and C3 are related to IgA nephropathy. It is generally considered that serum C3 levels show a normal range in patients with IgA nephropathy. Serum C3 levels in patients with IgA nephropathy were significantly lower than those in patients with non-IgA nephropathy including MPGN. In this study, serum IgA levels in patients with IgA nephropathy were significantly higher than those in patients with non-IgA nephropathy. Therefore, it appears that the serum IgA/C3 ratio may improve both diagnostic sensitivity and specificity. The results showed that the serum IgA/C3 ratio was a more useful marker than serum IgA to distinguish IgA nephropathy from non-IgA nephropa-

TABLE 4. Comparison of IgA nephropathy and non-IgA nephropathy

	Sensitivity	Specificity	Efficiency	Cut-off	Area
Serum IgA	74	54	67	250	0.67
Serum C3	70	50	62	125	0.61
Serum IgA/C3	79	61	73	2.14	0.71
Function	83	57	75	0.504	0.74

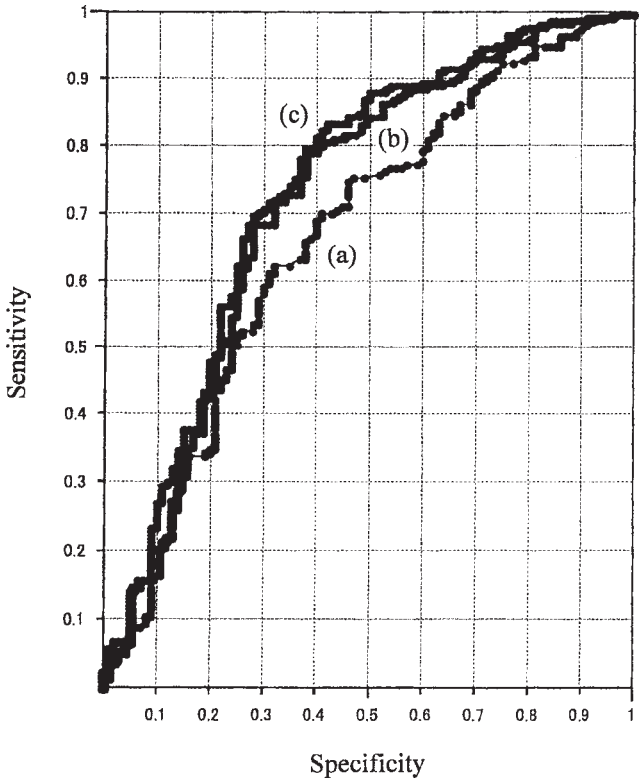

Fig. 1. Sensitivity and specificity of serum IgA, serum IgA/C3 and discriminant function. Receiver-operating characteristic plots were constructed to compare the diagnostic sensitivity and the diagnostic specificity of the serum IgA (a) and the serum IgA/C3 ratio (b). The discriminant function (c) was used for serum IgA and C3 to distinguish IgA nephropathy from non-IgA nephropathy as follows: Score = $0.023 \times C3 - 0.006 \times IgA - 0.791$.

thy. The discriminant function for serum IgA and C3 has the same diagnostic sensitivity and specificity as the serum IgA/C3 ratio. However, calculation of the discriminant function is more difficult than that of the serum IgA/C3 ratio. It appears that the serum IgA/C3 ratio is a more useful marker to distinguish IgA nephropathy from non-IgA nephropathy together with serum IgA levels.

The serum samples for this study were stored for a few months at –20°C or –70°C in each hospital. Therefore, the assay values of serum C3 using the stored samples might be greater than those of fresh samples. Around 30% higher results were obtained due to conversion of the complements as described previously (10). It is necessary to examine the serum IgA/C3 ratio using fresh samples. Although the gold standard for patients with IgA nephropathy is renal biopsy, general physicians can not perform biopsies easily. It appears that measurement of serum IgA and C3 may predict the diagnosis of patients with IgA nephropathy prior to renal biopsy.

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