Showa Univ J Med Sci **27**(3), 167～174, September 2015

Original

Prominent IgM Deposition in Glomerulus Is Associated with Severe Proteinuria and Reduced after Combined Treatment of Tonsillectomy with Steroid Pulse Therapy in Patients with IgA Nephropathy

Tomoaki MIYAZAKI*[1)], Kiyoko INUI[1)], Shinya OMIYA[1)], Sakura NAGUMO[2)], Nobuharu KANESHIMA[1)], Eri KAWASHIMA[1)], Yoshihiko INOUE[1)], Yuko YAMANO[2)], Toshio NAKADATE[2)] and Ashio YOSHIMURA[1)]

Abstract: IgA nephropathy (IgAN) is characterized by mesangial deposition of IgA, C3, and often IgM. We examined the relationship among IgM deposition, clinical features, and renal outcome in IgAN patients who underwent combined treatment of tonsillectomy with steroid pulse therapy (Tx-SP). We retrospectively reviewed 73 IgAN patients treated with Tx-SP from March 2006 to March 2014. The patients were divided into those with moderate (2+) to severe (3+) mesangial IgM deposition (Prominent IgM-positive patients, P-Group) and those with negative (−) to faint (1+) deposition (the "Other" patients, O-Group). Using propensity scores to minimize confounding factors, 11 propensity score-matched patients with O-Group (mO-Group) were compared to 11 P-Group patients. The study outcome was defined as urinary protein grade by urine test strip before Tx-SP and one year after Tx-SP. P-Group patients exhibited an increased severity of proteinuria compared to O-Group ($p = 0.018$) and mO-Group patients ($p = 0.009$) before Tx-SP. After Tx-SP, proteinuria was significantly ameliorated in the P-Group, reaching the same severity recorded in the O-Group ($p = 0.007$) and mO-Group ($p = 0.021$). No significant differences were noted between P-Group and mO-Group in microhematuria, serum creatinine level, and histological severity. Prominent IgM deposition is associated with severe proteinuria in IgAN. However, Tx-SP induces a sufficient reduction in the severity of proteinuria in IgM-positive IgAN.

Key words: IgA nephropathy, IgM deposition, proteinuria, tonsillectomy, steroid pulse therapy

Introduction

IgA nephropathy (IgAN) is the most common lesion causing primary glomerulonephritis in developed countries, and its only clinical manifestation is hematuria or proteinuria[1-8]. IgAN diagnosis can only be confirmed by kidney biopsy, where pathognomonic findings on immuno-

[1)] *Division of Nephrology, Department of Medicine, Showa University Fujigaoka Hospital,* 1-30 Fujigaoka, Aoba-ku, Yokohama 227-8501, Japan.
[2)] *Department of Hygiene and Preventive Medicine, Showa University School of Medicine.*
* To whom corresponding should be addressed.

fluorescence microscopy typically show prominent glomerular deposits of IgA (often accompanied by C3 and IgG) in the mesangium and along the glomerular capillary wall. Proteinuria is a serious risk factor of disease progression in IgAN patients[9]. While two general approaches have been developed to slow such progression – namely blood pressure control using angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers and therapy with glucocorticoids[10] – combined treatment of tonsillectomy with steroid pulse therapy (Tx-SP) has been reported to induce clinical remission of IgAN, representing a more promising, proactive therapy[11].

Despite progress, the indications for Tx-SP remain unclear. Differences in disease severity and the presence of multiple subtypes of IgAN have been observed, with some reports of patients with IgM-positive IgAN having more severe proteinuria than those with IgM-negative IgAN. Further study to corroborate these claims is necessary[12, 13].

Here, we compared the clinical features of Prominent IgM-positive (≥ 2＋ intensity) IgAN patients with other IgAN patients and evaluated the clinical response to Tx-SP treatment in both groups.

Methods

Study design and patients

Our retrospective cohort study was conducted from March 2006 to March 2014, and approved by the Showa University Fujigaoka Hospital Institutional Review Board.

A total of 119 patients aged 16 years or older were diagnosed with IgAN at Showa University Fujigaoka Hospital, and received a combined treatment of tonsillectomy with steroid pulse therapy (Tx-SP). IgAN was diagnosed based on light microscopy findings of mesangial proliferative changes, immunofluorescence (IF) findings of mesangial IgA and C3 deposition, and electron microscopy findings of electron-dense deposits in glomerular mesangial tissue obtained by renal biopsy. Of the initial 119 patients, 46 were excluded from the study due to more than a year lapsing between renal biopsy and Tx-SP or for having a history of systemic disease, such as diabetes mellitus, systemic lupus erythematosus, severe infection, or secondary IgAN. Clinical parameters of the 73 patients without missing data are shown in Table 1 and include age, sex, medication history, qualitative urinary protein (UP) level, urinary red blood cell (U-RBC) count, serum creatinine (sCr) levels, and estimated glomerular filtration rate [eGFR; calculated using the Modification of Diet in Renal Disease formula for Japanese. $eGFR = 186.3 \times sCr^{-1.154} \times Age^{-0.203}$ ($\times 0.742$ if female) $\times 0.881$).

Qualitative urinary protein level was converted to a score using a urine test strip as follows: negative / Grade 0, 1＋ / Grade 1, 2＋ / Grade 2, and ≥ 3＋ / Grade 3. U-RBC count was also converted into a score based on the number of red blood cells in a high-power field (x400) as follows: none–4 / Grade 0, 5–9 / Grade 1, 10–29 / Grade 2, and ≥ 30 / Grade 3. All clinical parameters were measured at two points. Baseline data were collected at the time of hospitalization for the purpose of renal biopsy (Before) and follow-up data were collected in the outpatient section at one year after the start of Tx-SP (After).

The Tx-SP protocol states that, for tonsillectomy, the tonsils are to be examined by an otorh-

Table 1. Clinical findings at the time of renal biopsy

Total number of patients, n	73
Age, years	35.1 ± 13.7
Sex (male / female, n)	47 / 26
medication, n (%)	
ACE Inhibitor	3 (4.1)
Angiotensin II receptor blockers	30 (41.1)
Dipyridamole	17 (23.3)
Dilazep	14 (19.2)
UP, grades (0 / 1 / 2 / 3, n)	25 / 22 / 22 / 4
U-RBC, grades (0 / 1 / 2 / 3, n)	18 / 12 / 27 / 16
S-Cr, mg / dl	0.93 ± 0.26
eGFR, ml / min / 1.73 m^2	75.56 ± 22.71

Values are presented as mean ± SD
ACE, angiotensin-converting enzyme; UP, qualitative urinary protein (Grade 0: trace, Grade 1: 1+, Grade 2: 2+, Grade 3: ≥ 3+); U-RBC, urinary red blood cell count in a high-power field (Grade 0: 0-4, Grade 1: 5-9, Grade 2: 10-29, Grade 3: ≥ 30); S-Cr, serum creatinine levels; eGFR, estimated glomerular filtration rate

inolaryngologist during surgery (after obtaining informed consent), regardless of the gross appearance of the tonsils and even in the absence of episodes of recurrent tonsillitis or gross hematuria with tonsillitis[14]. During the first year after tonsillectomy, Tx-SP should be administered as follows: high-dose methylprednisolone (0.5 g / day 3 times a week for 3 consecutive weeks), followed by oral prednisolone at an initial dose of 30 mg every other day, with gradual tapering of the dose over the course of 1 year. The study outcome was defined as urinary protein grade by urine test strip before Tx-SP and one year after Tx-SP.

Pathological analysis

Renal tissue samples obtained by percutaneous needle biopsy were divided into three portions, which were processed separately for study by light, IF, and electron microscopy, respectively, using standard techniques. For light microscopy, the renal tissue samples were fixed in 4 % buffered formaldehyde, embedded in Paraplast, and stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid methenamine silver, and Masson's trichrome. For IF microscopy, the renal tissue samples were frozen in liquid nitrogen, and cryostat sections were stained with fluorescein isothiocyanate-labeled antisera to human IgA, IgG1, IgG4, IgM, kappa and lambda light chains, C3, C1q, C4, and fibrinogen. The histologic severity was graded using Clinical Guides for IgA Nephropathy in Japan, third version[15]. Immunofluorescence staining level was converted into a score of immunoglobulin deposition in the mesangial matrix, as follows: Grade 0: − ～ ±, Grade 1: +, Grade 2: ++, Grade 3: +++.

Statistical analysis

We used a matched cohort approach based on the presence of IgM deposition in IgAN patients to minimize the influence of confounding factors. Propensity score matching is a method of adjusting for the observed characteristics of non-randomly assigned patients[16]. In this study, we matched the number of moderate (2+) to severe (3+) mesangial IgM deposition patients (collectively "Prominent IgM-positive patients") with negative (−) to faint (1+) mesangial IgM deposition patients (collectively the "other" patients) after calculating a propensity score for each patient. We derived the propensity score from a multilogistic regression model containing the following variables: age, sex, and medication history. Propensity scoring analysis was used to adjust for selection bias. In this way, patients were divided into three groups according to mesangial deposition: Prominent IgM-positive patients (P-Group: n = 11), the "Other" patients (O-Group: n = 62), and propensity score-matched patients (Prominent IgM-positive patients with the other patients; mO-Group: n = 11).

Results are expressed as either mean ± standard deviation or as a percentage. Non-parametric variables were compared using the Mann-Whitney U test, while categorical variables were compared using Fisher's exact test. *P*-values less than 0.05 were considered statistically significant. Results were analyzed using JMP Pro 11.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

The characteristics of the three groups are summarized and compared in Table 2. Age, sex, urine RBC, sCr, and eGFR were comparable among the three groups. However, UP grade was higher in the P-Group than in either the O-Group (UP Grade 0/1/2/3: 0/5/6/0 vs. 25/17/16/4, $p = 0.018$) or mO-Group (vs. 4/0/6/1, $p = 0.009$) before Tx-SP. After Tx-SP, UP grade in the P-Group decreased significantly, reaching the same grade as in the other patients (slope of urinary protein Grade −3/−2/−1/0/1: 0/3/8/0/0 vs. 2/12/17/29 in O-Group, $p = 0.007$; and vs. 0/5/2/4/0 in the mO-Group, $p = 0.021$).

Table 3 details the pathological features of the Prominent IgM-positive and matched patients. No marked histological differences were noted between the P-Group and mO-Group, including histological severity grade (1/2/3/4): 5/4/1/1 vs. 5/4/1/1, $p = 1.000$. There was also no significant difference between P-Group and mO-Group in the mesangial deposition of IgG and C1q, a parameter that has been associated with renal dysfunction in previous studies[17, 18].

Discussion

In the present study, we examined the effect of Tx-SP on proteinuria in IgAN patients independent of glomerular prominent IgM deposition levels. Prominent IgM deposition in the glomerulus was associated with severe proteinuria in patients with IgAN, despite a lack of marked differences in histological severity grade between patients with and without prominent IgM deposition. We also noted a decrease in urinary protein excretion in Prominent IgM-positive patients after Tx-SP to levels similar to those observed in the other patients. Persistent proteinuria is a risk factor for poor prognosis in renal function[9, 19], thus we expect that Tx-SP could improve the

Table 2. Comparison of clinical variables

		Unmatched cohort	p^*	Matched cohort	p^{**}
	P-Group (n = 11)	O-Group (n = 62)		mO-Group (n = 11)	
Age, years	34.8 ± 13.1	35.2 ± 13.9	0.865	35.7 ± 14.7	0.818
Sex (Male / Female)	6 / 5	41 / 21	0.506	6 / 5	1.000
UP, grades (0 / 1 / 2 / 3)					
Before	0 / 5 / 6 / 0	25 / 17 / 16 / 4	0.018	4 / 0 / 6 / 1	0.009
After	8 / 3 / 0 / 0	48 / 12 / 2 / 0	0.777	8 / 3 / 0 / 0	1.000
Δ UP (−3 / −2 / −1 / 0 / 1)	0 / 3 / 8 / 0 / 0	2 / 12 / 17 / 29 / 2	0.007	0 / 5 / 2 / 4 / 0	0.021
U-RBC, grades (0 / 1 / 2 / 3)					
Before	3 / 1 / 5 / 2	15 / 11 / 22 / 14	0.886	1 / 2 / 7 / 1	0.643
After	9 / 2 / 0 / 0	52 / 4 / 5 / 1	0.458	9 / 2 / 0 / 0	1.000
Δ U-RBC (−3 / −2 / −1 / 0 / 1 / 2)	1 / 5 / 2 / 3 / 0 / 0	10 / 19 / 15 / 16 / 1 / 1	0.917	1 / 5 / 4 / 1 / 0 / 0	0.712
sCr, mg / dl					
Before	0.87 ± 0.26	0.94 ± 0.26	0.287	0.93 ± 0.28	0.450
After	0.83 ± 0.21	0.93 ± 0.26	0.257	0.92 ± 0.26	0.576
Δ sCr	−0.04 ± 0.12	−0.02 ± 0.18	0.589	−0.01 ± 0.17	0.768
eGFR, ml / min / 1.73 m^2					
Before	79.35 ± 23.06	74.88 ± 22.77	0.469	72.20 ± 17.46	0.450
After	81.94 ± 26.71	75.20 ± 20.94	0.611	72.33 ± 20.38	0.375
Δ eGFR	2.59 ± 14.09	0.31 ± 17.49	0.568	0.13 ± 17.78	0.622

P-Group, IgA nephropathy patients with moderate (2+) to severe (3+) mesangial IgM deposition; O-Group, IgA nephropathy patients with negative (−) to faint (+) mesangial IgM deposition; mO-Group, matched IgA nephropathy patients with negative (−) to faint (+) mesangial IgM deposition; UP, urinary protein qualitative (Grade 0: trace, Grade 1: 1+, Grade 2: 2+, Grade 3: ≥3+); U-RBC, urinary red blood cell sediments / high-power field (Grade 0: 0–4, Grade 1: 5–9, Grade 2: 10–29, Grade 3: ≥30); sCr, serum creatinine; eGFR, estimated glomerular filtration rate; $^*p$-value for the 62 unmatched other patients vs. the Prominent IgM-positive patients; $^{**}p$-value for the 11 matched other patients vs. the Prominent IgM-positive patients.

renal prognosis of Prominent IgM-positive IgAN patients.

IgAN is understood to have multiple subtypes; for example, deposition of IgG or IgM is not believed to affect renal prognosis in IgAN patients[20], and children with IgAN found to be positive for IgM or C1q at biopsy can show severe proteinuria[12]. Previous studies have widely defined IgM deposition as a score of ≥1+[12]. However, herein we defined IgM deposition of 2+ to 3+ as "Prominent IgM-positive", representing the high-level deposition group. In this way, we could clearly draw a link between high IgM deposition and proteinuria, as an IgM deposits in the mesangium region of ≥2+ was listed among the definitive findings of IgM nephropathy (IgMN)[21]. IgMN is glomerulonephritis with proteinuria, similar to IgAN, but without all the diagnostic indications of IgAN, and IgMN has been associated with nephrotic syndromes such as minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS)[22,23].

We suspect that Prominent IgM-positive IgA nephropathy patients may be at risk of developing not only size selectivity disorder due to glomerulus capillaritis, but also charge selectivity

Table 3. Histological appearance at time of renal biopsy

	P-Group (n＝11)	mO-Group (n＝11)	*p*
H-Grade (1 / 2 / 3 / 4)	5 / 4 / 1 / 1	5 / 4 / 1 / 1	1.000
Immunofluorescence staining Grade (0 / 1 / 2 / 3)			
IgG	7 / 3 / 1 / 0	8 / 3 / 0 / 0	1.000
IgA	0 / 9 / 2 / 0	2 / 8 / 1 / 0	0.587
IgM	0 / 0 / 11 / 0	4 / 7 / 0 / 0	＜0.001
Fbg	4 / 6 / 1 / 0	7 / 4 / 0 / 0	0.395
C3	1 / 2 / 8 / 0	0 / 1 / 10 / 0	0.587
C1q	9 / 2 / 0 / 0	10 / 1 / 0 / 0	1.000
C4	9 / 2 / 0 / 0	10 / 1 / 0 / 0	1.000
kappa	2 / 6 / 3 / 0	2 / 8 / 1 / 0	0.825
lambda	0 / 1 / 9 / 1	1 / 4 / 6 / 0	0.220

P-Group, IgA nephropathy patients with moderate (2＋) to severe (3＋) mesangial IgM deposition; mO-Group, matched IgA nephropathy patients with negative (－) to faint (＋) mesangial IgM deposition; H-Grade, glomeruli with lesions associated with renal prognosis / total number of glomeruli (Grade 1: 0%-24.9%, Grade 2: 25%-49.9%, Grade 3: 50%-74.9%, Grade 4: ≥75%)
Immunofluorescence staining Grade is deposition of immunoglobulin in mesangial matrix (Grade 0: － ～ ±, Grade 1: ＋, Grade 2: ＋＋, Grade 3: ＋＋＋)

disorder, as recorded with nephrotic syndromes such as MCD and FSGS. These suspicions are based on the fact that initial proteinuria showed the only difference in clinical parameters and degree of histological damage on renal biopsy between the P-Group and mO-Group in the present study.

Several limitations to our study warrant mention. First, proteinuria was evaluated qualitatively, rather than quantitatively, due to quantitative data not being measured at all time points based on the physician's decision. Urinary protein quantitative data are generally measured at the time of hospitalization for the purpose of renal biopsy, but only rarely in outpatients after Tx-SP. Therefore we could not use the data we had in this analysis (Table 4). Second, we only assessed the presence of glomerular lesions in our histological evaluation, not framework disorder, as our histological evaluation referenced renal biopsy evaluation results and was conducted by the attending physician based on medical records, thereby preventing observer bias. Oxford categorization criteria were established in 2009, and no stromal evaluation was conducted before that point. We therefore used "Clinical Guides for IgA Nephropathy in Japan, third version"[15] to describe histological disease severity. Third, Tx-SP was performed in this study, but steroid pulse therapy alone might be effective. We believe that the severe proteinuria observed in Prominent IgM-positive IgAN is due to the presence of charge selectivity disorder, similar to that observed with nephrotic syndrome. Because steroid therapy itself is a treatment modality of nephrotic syndrome, tonsillectomy may not be necessary. Future investigations should take these three points into account.

Table 4. Comparison of urine protein quantitation (g / gCr)

	P-Group (n = 11)	O-Group (n = 62)	p^*
Before	0.961 ± 0.788	0.429 ± 0.553	0.014
n. / total n.	11 / 11	61 / 62	
After	0.268 ± 0.169	0.165 ± 0.273	0.064
n. / total n.	5 / 11	20 / 62	
Δ UP	−0.856 ± 0.613	−0.543 ± 0.755	0.213
n. / total n.	5 / 11	19 / 62	

P-Group, IgA nephropathy patients with moderate (2+) to severe (3+) mesangial IgM deposition; O-Group, IgA nephropathy patients with negative (−) to faint (+) mesangial IgM deposition; UP, urinary protein; p-value for the 62 unmatched patients vs. the Prominent IgM-positive patients.

In conclusion, prominent IgM deposition in glomerulus is associated with severe proteinuria in patients with IgAN. Our study confirms that Tx-SP induces a marked reduction of proteinuria severity in Prominent IgM-positive IgAN patients and may therefore represent a powerful therapeutic strategy in this particular patient population.

Acknowledgment

The authors would like to thank DMC (dmed.co.jp) for English editing.

Conflict of interest disclosure

The authors have neither financial support nor relationships to disclose.

References

1) Wyatt RJ, Julian BA. IgA nephropathy. *N Engl J Med.* 2014;**368**:2402-2414.
2) D'Amico G. Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. *Semin Nephrol.* 2004;**24**:179-196.
3) Emancipator SN. IgA nephropathy: morphologic expression and pathogenesis. *Am J Kidney Dis*. 1994;**23**:451-462.
4) Galla JH. IgA nephropathy. *Kidney Int*. 1995;**47**:377-387.
5) Donadio JV, Grande JP. IgA nephropathy. *N Engl J Med*. 2002;**347**:738-748.
6) Li LS, Liu ZH. Epidemiologic data of renal diseases from a single unit in China: analysis based on 13,519 renal biopsies. *Kidney Int.* 2004;**66**:920-923.
7) Simon P, Ramee MP, Boulahrouz R, *et al*. Epidemiologic data of primary glomerular diseases in western France. *Kidney Int*. 2004;**66**:905-908.
8) Barratt J, Feehally J. IgA nephropathy. *J Am Soc Nephrol*. 2005;**16**:2088-2097.
9) Le W, Liang S, Hu Y, *et al*. Long-term renal survival and related risk factors in patients with IgA nephropathy: results from a cohort of 1155 cases in a Chinese adult population. *Nephrol Dial Transplant.* 2012;**27**:1479-1485.
10) Chapter10:Immunoglobulin A nephropathy.*Kidney Int Suppl (2011)*. 2012;**2**:209-217.
11) Hotta O, Miyazaki M, Furuta T, *et al*. Tonsillectomy and steroid pulse therapy significantly impact on clinical

remission in patients with IgA nephropathy. *Am J Kidney Dis*. 2001;**38**:736-743.
12) Welch TR, McAdams J. Immunoglobulin M and C1q mesangial labeling in IgA nephropathy. *Am J Kidney Dis*. 1998;**32**:589-592.
13) Liu Z, Li L. Significance of IgM deposition in IgA nephropathy : an appraisal based on renal re-biopsy. *Zhonghua Yi Xue Za Zhi*. 1990;**70**:324-326,24. (in Chinese).
14) Ieiri N, Hotta O, Sato T, *et al*. Significance of the duration of nephropathy for achieving clinical remission in patients with IgA nephropathy treated by tonsillectomy and steroid pulse therapy. *Clin Exp Nephrol*. 2012;**16**:122-129.
15) Matsuo S, Kawamura T, Yasuda T, *et al*. Clinical guides for immunoglobulin A (IgA) nephropathy in Japan, third version. Jpn J Nephrol.2011;**53**:123-135. (in Japanese).
16) D'Agostino RB Jr. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. *Stat Med*. 1998;**17**:2265-2281.
17) Nishiwaki H, Hasegawa T, Nagayama Y, *et al*. Absence of mesangial c1q deposition is associated with resolution of proteinuria and hematuria after tonsillectomy plus steroid pulse therapy for immunoglobulin a nephropathy. *Nephron*. 2015;**130**:1-7.
18) Wada Y, Ogata H, Takeshige Y, *et al*. Clinical significance of IgG deposition in the glomerular mesangial area in patients with IgA nephropathy. *Clin Exp Nephrol*. 2013;**17**:73-82.
19) Reich HN, Troyanov S, Scholey JW, *et al*. Remission of proteinuria improves prognosis in IgA nephropathy. *J Am Soc Nephrol*. 2007;**18**:3177-3183.
20) Moriyama T, Shimizu A, Takei T, *et al*. Characteristics of immunoglobulin A nephropathy with mesangial immunoglobulin G and immunoglobulin M deposition. *Nephrology*. 2010;**15**:747-754.
21) Neeraja K. IgM Nephropathy. In *Colvin Robert B. eds. Diagnostic pathology. Kidney diseases*. [Salt Lake City] Amirsys ; 2011. pp 44-45.
22) Border WA. Distinguishing minimal-change disease from mesangial disorders. *Kidney Int*. 1988;**34**:419-434.
23) Korbet SM. Primary focal segmental glomerulosclerosis. *J Am Soc Nephrol*. 1998;**9**:1333-1340.

[Received May 23, 2015 : Accepted June 25, 2015]