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Reflux Nephropathy and Glomerular Sclerosis: Glomerular Hypertrophy as a Possible Result of Remnant Glomerular Hyperperfusion and Hypertension

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Summary. **We reported the clinicopathologic findings of 17 children with reflux nephropathy (RN) and the results of morphometric procedure for glomerular hypertrophy. The patients, including 13 boys and 4 girls, consisted of 8 with primary vesicoureteral reflux (VUR) and 9 with secondary VUR. Impaired renal function at renal biopsy (Ccr $<$ 50 ml/min/1.73 m^2) were observed in 12 patients. The latest renal status presents end-stage renal disease in 8 and progressive renal function deterioration in 4 patients. Although 4 patients showed end stage kidney, glomerular changes in 7 out of 13 patients showed focal glomerular sclerosis (focal and segmental-glomerular sclerosis: 3, focal and global obsolescence: 4) with moderate to severe tubulo-interstitial changes. Dysplastic lesion and Tamm-Horsfall glycoprotein in the dilatated tubular lumens or interstitial area were observed in 2 patients. A significant glomerular hypertrophy was evident in patients with diminished renal function and segmental/global glomerular sclerosis.**

These results suggest that nephron mass reduction due to renal scarring in RN leads to remnant glomerular overload, glomerular hyperperfusion or hypertension, and results in proteinuria and glomerular injury progressing to glomerular sclerosis.

INTRODUCTION

Reflux nephropathy (RN), originally defined by Bailey[1] as renal parenchymal damage or scarring associated with vesicoureteral reflux (VUR), shows a progressive impairment of renal function in children and young adults, despite aggressive medical or surgical treatment. Therefore, the clinicopathological details and a possible pathogenesis of this disease are needed for it to be identified as soon as possible. Recently, a nonimmunological process leading to end-stage renal failure[3] has been highlighted because it is difficult to explain the progressive functional deterioration thoroughly by immunological phenomena in various glomerular diseases. We examined the clinicopathological findings of the cases of 17 children with RN and the possible relationship between glomerular hypertrophy and segmental/global glomerular sclerosis by using a histomorphometric procedure.

MATERIALS AND METHODS

Patients

Kidney tissues obtained from 17 children with RN were examined by histological, immunohistochemical, and morphometric light microscope study. The children (13 boys and 4 girls) were between 3 months and 14 years of age at the time of kidney biopsy or nephrectomy. There were 8 children with primary VUR, a congenital condition resulting mainly from a deficiency of anatomical structures of uretero-vesical junction, and 9 with secondary VUR, resulting from a mechanical or functional obstruction in the lower urinary tract such as the posterior urethral valve or neurogenic bladder. VUR was graded according to the International Classification system[4] (Fig. 1).

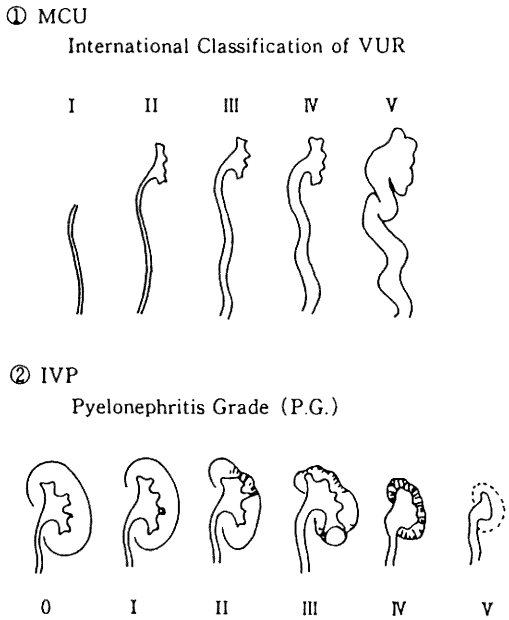

Fig. 1. International classification of VUR and Ikoma's pyelonephritis grade.

Twelve out of 17 patients had severe VUR, above grade III. To morphologically grade RN on intravenous pyelography (IVP), we used the Ikoma's pyelonephritis grade (PG) under the following criteria[2] (Fig. 1): grade 0—no changes; grade I—deformity confined to calyces or calyceal papillae; grade II—not more than two scarred areas; grade III—more than three scarred areas with some normal area; grade IV—generalized scarring; and grade V-end-stage shrunken kidney.

Renal functional deterioration, defined as less than 50 ml/min/1.73m² measured by endogenous creatinine clearance (Ccr), was observed in 12 patients at the time kidney tissue was obtained.

Glomerular morphometric study

We prepared serial histological sections ranging from 30 to 50 for light microscopic qualitative and quantitative study. At least 3 glomeruli for each patient, judged as a cross section of the glomerular equator, were used for glomerular morphometry with an image analyzer (QTM720, Cambridge company, England). The serial sections were stained with periodic acid-Schiff (PAS). Quantitative measurement was performed on each Bowman's capsular area (BM area), glomerular tuft area (Gl area), and the ratio of Bowman's capsular area/glomerular area (BM/Gl ratio, %). Two hundred and two glomeruli from 48 children, 22 boys and 26 girls, 2-19 years old, with normal renal function (Ccr>80 ml/min/1.73m², and minimal change nephrotic syndrome or isolated microscopic hematuria (less than 30/visual field), served as control glomeruli, because BM area, Gl area, and BM/Gl ratio showed no statistical significance between the minimal change nephrotic syndrome group and the isolated microscopic hematuria group. We also performed a morphometrc study on 25 children with serial biopsied IgA nephropathy and 15 children with primary focal and segmental glomerulosclerosis (FSGS), maintaining normal renal function at the time of renal biopsy. The morphometric study was applied on glomeruli with minimal change from RN or primary FSGS patients, and on glomeruli with minimal or slightly mesangial proliferative glomeruli from IgA nephropathy patients. To avoid age-related influences on the glomerular morphometric study, we used the Z score, known as the standard deviation score (SD score), calculated as follows; (mean—measured value)/SD for appropriate age.

The results were subjected to Student's t-test and considered statistically significant if $p<0.05$.

Immunohistochemical procedures

Kidney tissues were snap-frozen in pre-cooled liquid n-hexane and cut into 4 μm thick sections with a cryostat. FITC-labeled antihuman IgG, IgA, IgM, C3c sera (purchased from Behringwerke) and C3d (from DAKO) were reacted and incubated at 37°C for 30 min. Monoclonal antibodies against Type III, IV, and V collagens (kindly provided by Prof. A. Ooshima, Wakayama Prefectural Medical School, Wakayama, Japan) were first reacted with snap-frozen 4 μm thick sections, followed by incubation with FITC or TRITC-conjugated anti-mouse IgG (from Cappel) for Type III and IV collagen, and with FITC-conjugated anti-mouse IgM (from Cappel) for Type V collagen. FITC-conjugated anti-human uromucoid serum (from Behringwerke) were also reacted with paraffin-embedded sections, deparaffinized and 1% trypsin (type III, Sigma)-pretreated at 37°C for 60 min.

RESULTS

Clinicopathological features of reflux nephropathy (Tables 1, 2)

More than half of the patients with primary RN had progressed to end-stage kidney disease and complicated hypertension and proteinuria. Light micro-

Table 1. Clinicopathological features of reflux nephropathy due to primary VUR

Name	Age (years)	Prog-nosis	HT	Prote-inuria	PG r/l	VUR r/l	#of gl.	Proli-ferat.	seg scl	glob scl	T-I change infil/fib & atr	gl. change	others
G. H	0.6	←→	(−)	(−)	III/III	III/III	23	+*	0	+	+++/+++	FGO	imm gl
O. S	14	HD	+	+	IV/IV	III/III	81	0	+(h,p)	+++	+++/+++	ESKid	THP, dyspla
N. M	13	↘	+	+	III/III	III/III							
						scar(−)	6	0	0	0	0/0	minimal	
						scar	13	0	++(h)	+	++/++	FSGS	
						cort/med	6	+	++(h)	+	++/+++	FSGS	foam cell
M. T	14	↘	+	+	II/IV	II/III	3	0	0	0	0/0	minimal	
M. Y	13	↔	(−)	+	III/III	0/I							
						scar(−)	2	0	0	0	+/0	minimal	
						scar	8	0	+(?)	+++	+/++	FSGS	
K. K	9	HD	+	+	hypo (0/0)	II/II	5	0	++ (h,p)	0	+/+++	FSGS	
K. H	7	CRF	+	+	hypo (I/I)	II/0	1	0	0	0	+/++	minimal	
K.H	14	HD	+	+	hypo (0/0)	III/III	64	+	+(h)	+++	+++/+++	ESKid	THP
S. H	7	CRF	(−)	+	II/III	II/III	57	+	+(?)	+++	+++/+++	ESKid	
N. H	14	↘	(−)	+	III/II	III/IV							
						scar(−)	6	+	+(h)	+	0/0	FSGS	
T. H	11	HD	+	+	IV/IV	IV/III	20	+	++ (h,p)	+++	+++/+++	ESKid	

HT: hypertension, PG: pyelonephritis grade×seg/glob, scl: segmental/global sclerosis, T-I change: tubulo-interstitial, HD: hemodialysis, +*: seg proliferation, FGO: focal global sclerosis, ESKid: end stage kidney, imm gl: immature glomeruli, infil/fib & atr: mononuclear cell infiltration/fibrosis & tubular atrophy, THP: Tamm-Horsfall protein, (h,p): hilar, peripheral, dyspla: dysplasia, ↔: sustained normal renal function, ↘: progressive renal functional deterioration, cort/med: corticomedullary junction, CRF: chronic renal failure

scopic findings showed segmental glomerular sclerosis in 5 out of 8 patients, and moderate-to-severe tubulo-interstitial changes in 7 patients. In the unscarred area of the biopsy specimens, obtained from patients N. M. and M. Y., the glomerular lesions were minimal even though the glomeruli were serially examined through 50 sections by light microscopy. In the scarred or cortico-medullary area, segmental glomerular sclerosis was observed in 5 patients (6 specimens). The segmental sclerosis was mainly located near the vascular pole area in 2 patients (3 specimens), in both the vascular pole and peripheral glomerular tuft area in 2 patients (2 specimens), and unidentified in 1 patient. The area of segmental sclerosis was negative with colloidal iron stain, suggesting a reduction in the sialic acid content (Fig. 2). In patient O.S., nephrectomized prior to kidney transplantation, glomeruli of the renal cortical region had minimal glomerular alteration and segmental glomerular sclerosis, while most glomeruli in the deep cortical region of the corticomedullary junction exhibited global glomerular sclerosis (Fig. 3), and antibodies to IgG, IgM, C3c, and C3d fixed intensely to peripheral capillary loops and mesangium (Fig. 4). Other than the glomerular findings, striated PAS positive materials were found in two patients in tubular lumens (Fig. 5) or interstitium and were labeled with FITC-labeled anti-human uromucoid serum. Foam cells were observed in interstitium in 2 patients. As adhesions of glomerular tufts to Bow-

Table 2. Clinicopathological features of reflux nephropathy due to secondary VUR

Name	Age (years)	Prog-nosis	HT	Prote-inuria	PG r/l	VUR r/l	#of gl.	Proli-ferat.	seg scl	glob scl	T-I change infil/fib & atr	gl change	others
Y. J	5	↘	(−)	±	IV/IV	IV/IV	15	+*	0	++	++/++	FGO	
N. M	8	HD	+	+	III/IV	IV/IV	10	/	/	+++	+++/+++	ESKid	
U. K	0.3	↔	(−)	(−)	IV/IV	III/III	18	+*	0	+++	++/+++	FGO	imm gl
K. K	4	HD	(−)	+	IV/V	IV/IV	4	+*	0	+	++/+++	FGO	
N. Y	2	acci. death	(−)	(−)	III/IV	I /III	65	0	0	0	0/±	minimal	
K. M	6	HD	+	+	IV/ age.	III/−	26	0	0	+	+/+	FGO	
M. H	0.3	?	(−)	(−)	−/V	−/III	7	0	0	0	0/±	minimal	dyspla
T. K	3	HD	+	+	IV/IV	II/II	0	/	/	/	+++/+++	ESKid	
K. K	5	↘	(−)	(−)	IV/IV	III/III	40	0	0	0	0/0	minimal	

HT: hypertension, PG: pyelonephritis grade, seg/glob scl: segmental/global sclerosis, T-I change: tubulo-interstitial, HD: hemodialysis, +*: seg proliferation, FGO: focal global sclerosis, ESKid: end stage kidney, imm gl: immature glomeruli, infil/fib & atr: mononuclear cell infiltration/fibrosis & tubular atrophy, THP: Tamm-Horsfall protein, (h,p): hilar, peripheral, age: agenesis, dyspla: dysplasia, ↔: sustained normal renal function, ↘: progressive renal functional deterioration

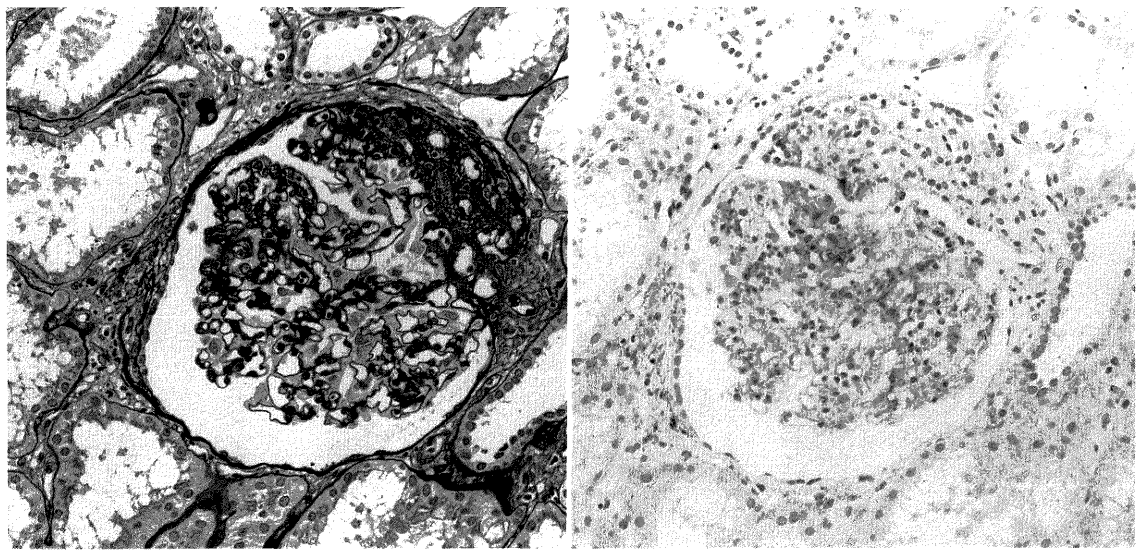

Fig. 2. Segmental glomerular sclerosis can be seen in the vascular pole region with PAS stain (left), whereas it showed negative stain with colloidal iron stain (right). Serial sections, ×200.

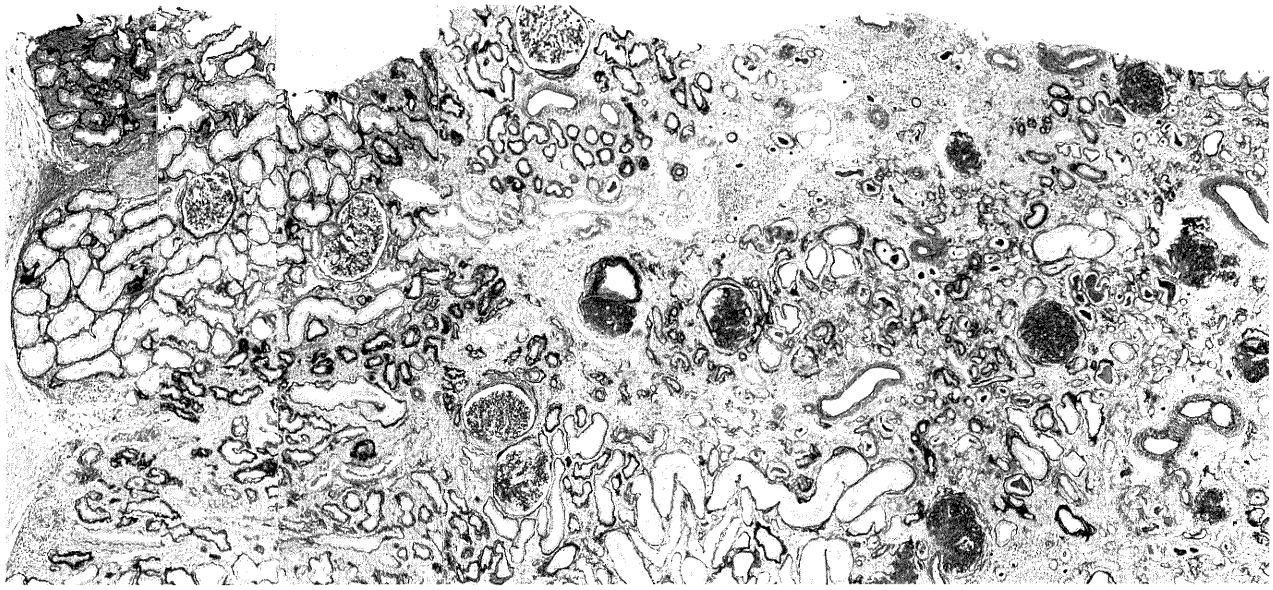

Fig. 3. Glomeruli of renal outer cortical region revealed minimal glomerular alterations or segmental glomerular sclerosis, while almost all glomeruli of deep cortical to corticomedullary junction exhibited global glomerular sclerosis in case O.S. (stained with PAS, ×40).

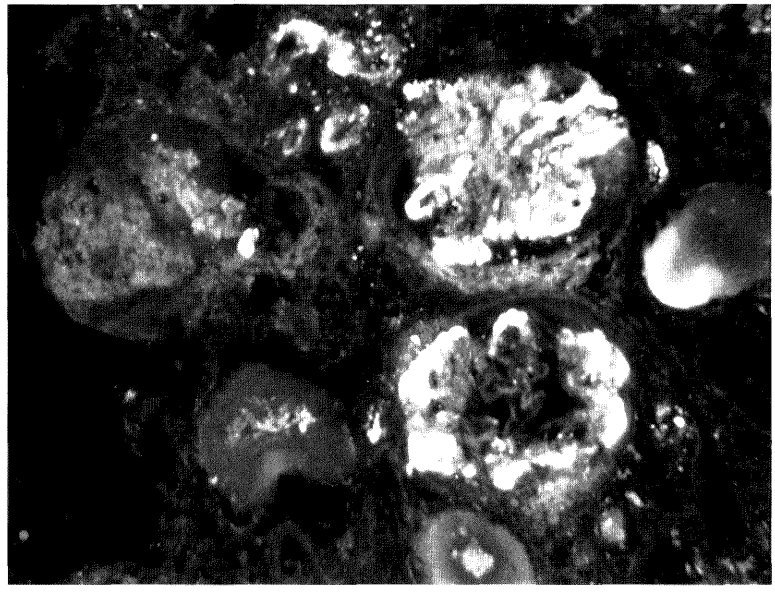

Fig. 4. Antibody serum to human C3c fixed particularly intensely to peripheral capillary loop, and less intensely to mesangial area in the right two glomeruli. However, the left glomerulus, completely hyalinized, had less deposition of C3c.

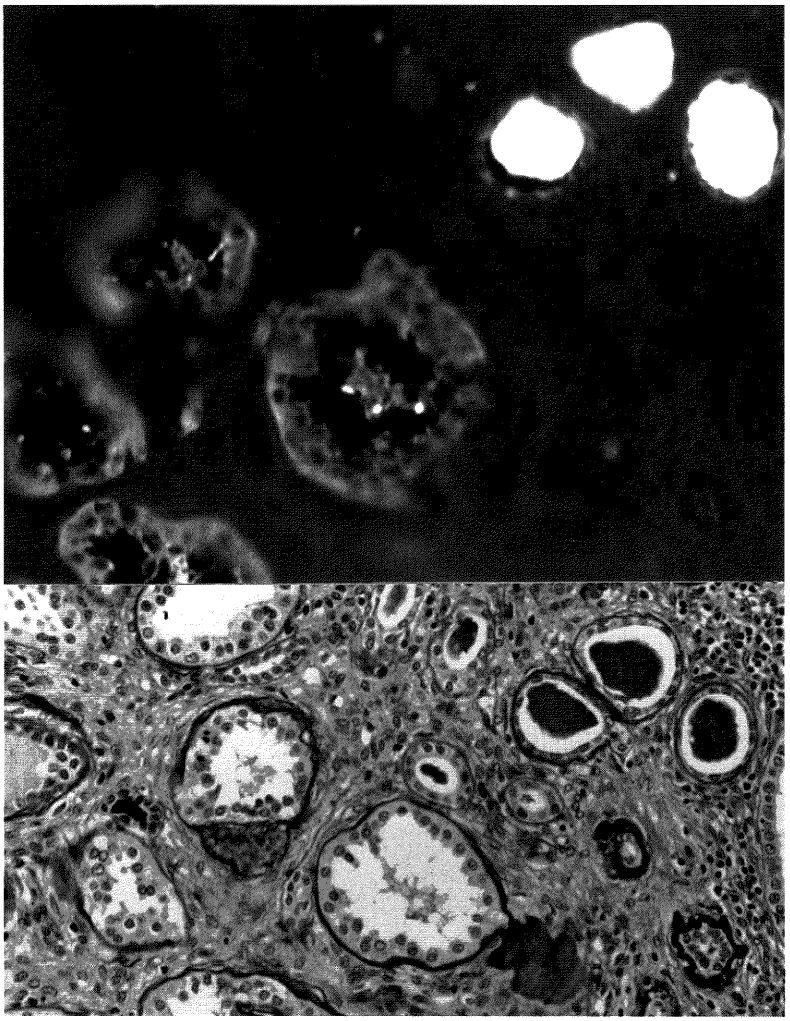

Fig. 5. Uromucoid can be detected in striated PAS-positive materials within tubular lumens and in the cytoplasm of epithelial cells in the distal tubules.

man's capsule frequently coexist with glomerular sclerosis in primary FSGS, we extensively searched the adhesions and glomerular sclerosis by preparing a series of serial sections. The lesion of adhesion, which contains swollen Bowman's epithelial cells or glomerular epithelial cells with vacuoles or protein-absorbed lysosomes in their cytoplasm in a section, switched over to segmental glomerular sclerosis with hyaline deposits in another section (Fig. 6).

Compared to primary VUR, the onset age of secondary VUR was lower and the clinical outcome was poorer. Most secondary VUR patients had severer grades of VUR and kidney scarring than primary VUR patients. Glomerular change in secondary VUR was mainly global sclerosis, although tubulo-interstitial changes were slightly similar to primary VUR. Other findings included premature glomeruli and dysplastic lesions.

In a normal kidney, antisera to Type IV collagen reacted with the glomerular basement membrane (GBM) and mesangium as well as the tubular basement membrane (TBM) and Bowman's capsule. Type III collagen was observed only in the interstitium and blood vessels, but it was not found in any part of the glomeruli. Type V collagen also located in the interstitium in addition to the regions where Type IV collagen reacted positively. In kidney tissue obtained from patient O.S., the intensity of fluorescence with

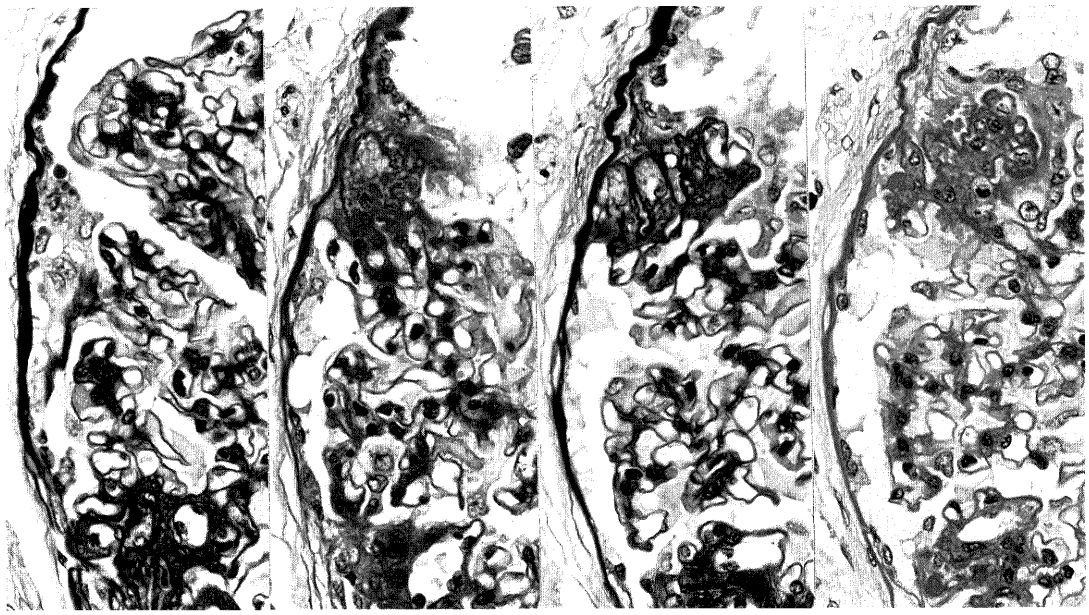

Fig. 6. Observation of adhesion to Bowman's capsule and hyaline deposits in a series of light microscopic sections stained with PAS, ×200.

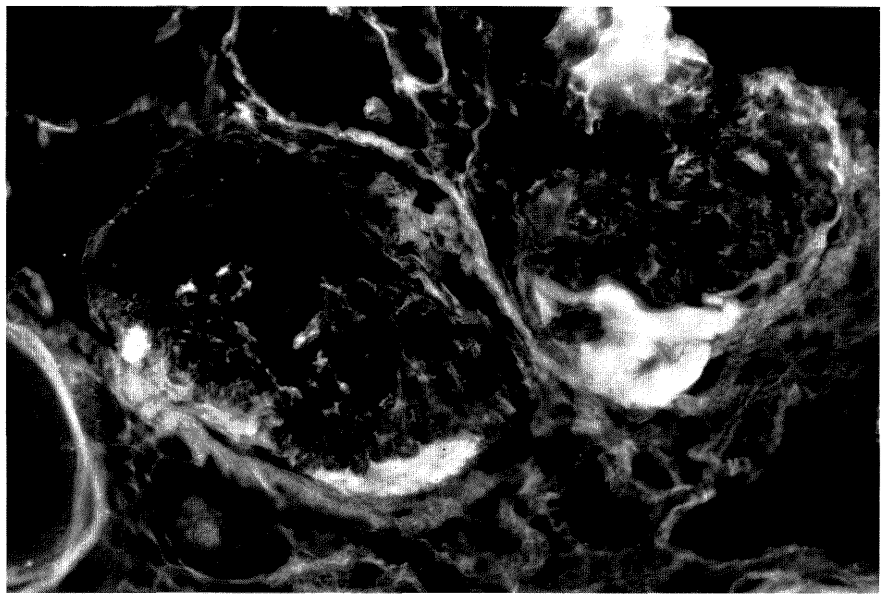

Fig. 7. Type III collagen. Intraglomerular and interstitial staining are prominent. Segmental reactivity with the glomerulus can be also seen (×200).

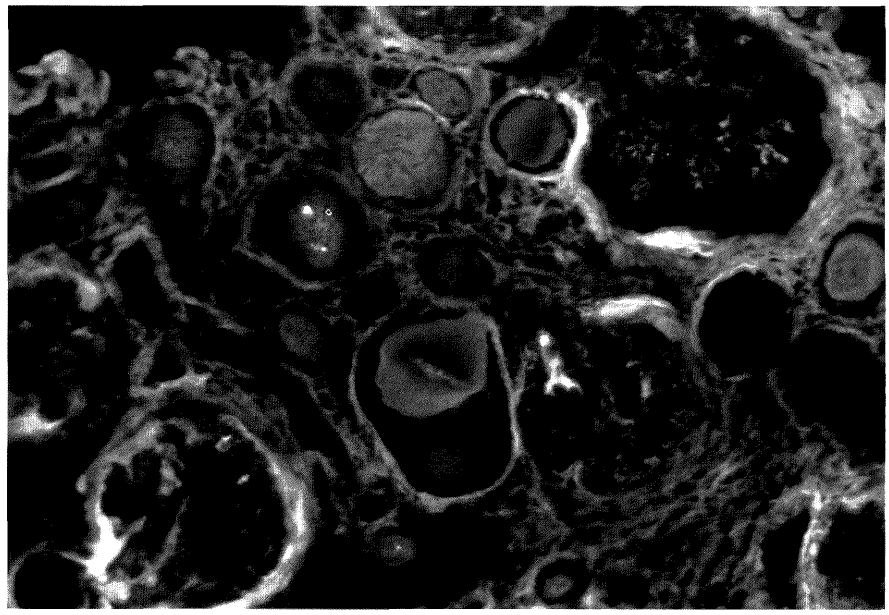

Fig. 8. Type V collagen. In three glomeruli, nearly hyalinized, GBM and mesangium are weakly positive, but periglomerular fibrosis and intraglomerular sclerosis are intensely positive (×100).

antibody to Type IV collagen in nearly or totally hyalinized glomeruli diminished markedly. In the glomeruli with an increase of mesangial matrix, staining for Type III and V was intense. Periglomerular fibrosis showed intense staining for Type III, IV, and V collagen (Figs. 7, 8).

Glomerular morphometric study

Glomerular morphometric data in children with minimal change nephrotic syndrome and isolated microscopic hematuria were summarized in Table 3. Both the BM and Gl areas enlarged with age, while the BM/Gl ratio decreased with age.

There was a significant enlargement of the glomerular tuft area of RN with a decrease in Ccr less than 60 ml/min/1.73m², although 3 out of 4 patients with Ccr above 60 ml/min/1.73m² showed no significant enlargement (Fig. 9). The glomerular tuft areas of 5 patients (54,834±3,310 μm^2, mean±SD), aged 11 to 15, showed statistically significant enlargement in comparison to the control group (14,037±2,663 μm^2, n=24), while Bowman's capsular area (75,664±6,737 μm^2, n=5) showed no significant enlargement (Fig. 10), as compared to the control (20,629±3,319 μm^2). The glomerular hypertrophy was not influenced by the presence of the scarred region. Although glomerular hypertrophy was evident in glomeruli showing minimal or slight-to-mild proliferative change, it was not apparent in glomeruli with moderate-to-severe proliferation, synechiae, and global sclerosis.

The BM/Gl ratio (%, Fig. 11) of 5 patients (137.0±5.0%), aged 11 to 15, was significantly smaller than that of age-matched controls (148.4±9.7%), indicating that glomerular hypertrophy resulted from the enlargement of the glomerular tuft area rather than that of Bowman's capsular area.

Regarding the relationship between Ccr and the SD score calculated by glomerular morphometric study in RN, there was a rapid increase in the SD score of the BM and Gl areas when Ccr diminished to less than 60 ml/min/1.73m² (Fig. 12.).

The changes in the glomerular tuft area of the serially biopsied 25 children with IgA nephropathy maintaining normal renal function (Ccr>80 ml/min/1.73m²), were parallel to corresponding changes in the control group. However, among 3 patients who showed glomerular hypertrophy beyond +1SD, 1 patient, whose biopsies were performed at 12 and 14 years of age, had progressive functional deterioration (serum creatinine: 1.4 mg/dl, Ccr=60 ml/min/1.73m²) 4 years after the second biopsy (Fig. 13). The change of Bowman's capsular area was nearly the same.

BM and Gl areas and the BM/Gl ratio in primary FSGS showed a significant difference compared to the control group or minimal change nephrotic syn-

Table 3. Glomerular morphometric data in children with minimal change nephrotic syndrome and isolated microscopic hematuria. (N = 48 children, 202 gl.)

Age (yr)	# case	# of Gl	BM area	BM-SD	Gl area	Gl-SD	BM/Gl (%)	BM/Gl-SD
2	2	10	9371	1897	6076	1271	156	17
4	2	9	12713	1980	7710	1438	166	17
5	3	14	11360	1480	7701	1322	149	14
6	1	5	15182	2859	9927	1622	153	13
7	5	17	14272	2343	9241	1483	153	13
8	1	5	15698	1117	10045	817	156	3
9	3	15	17544	4576	12004	3042	147	12
10	4	14	19039	3692	13347	3015	143	12
11	9	45	18479	3474	12061	1950	153	15
12	3	15	20247	4469	13261	2967	153	10
13	5	21	20118	3657	14081	2553	143	11
14	5	14	26101	5363	18593	4139	142	11
15	2	5	20694	2274	14868	1661	141	6
16	2	9	23408	3711	17880	3363	132	8
19	1	4	23458	2652	17678	2653	133	6

BM area: Bowman's capsular area (μm^2)
Gl area: Glomerular tuft area (μm^2)
BM/Gl (%): Bowman/Glomerular ratio (%)
SD: Standard deviation

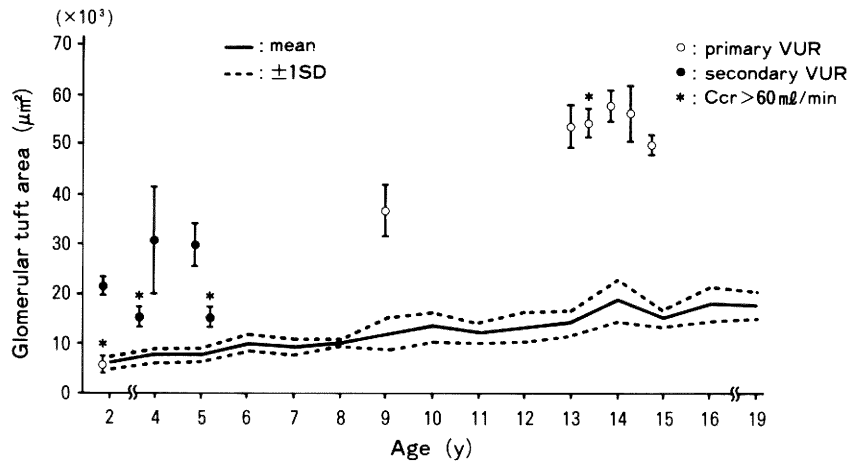

Fig. 9. Morphometric measurement of glomerular tuft area in children with reflux nephropathy.

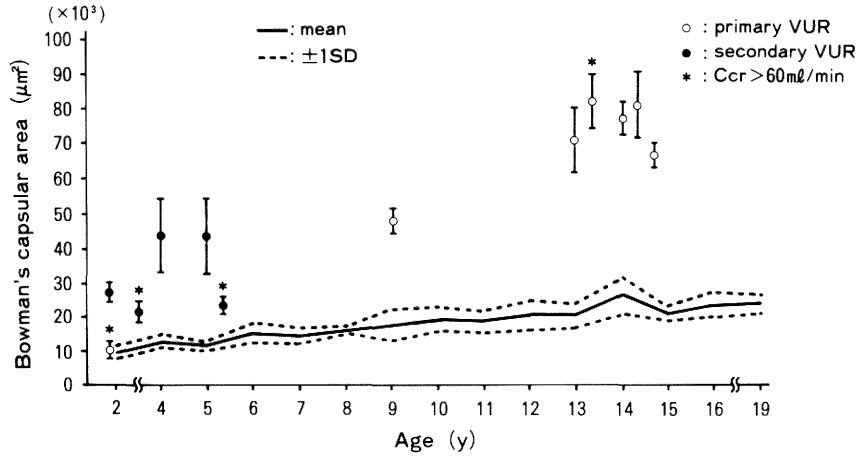

Fig. 10. Bowman's capsular area in children with reflux nephropathy.

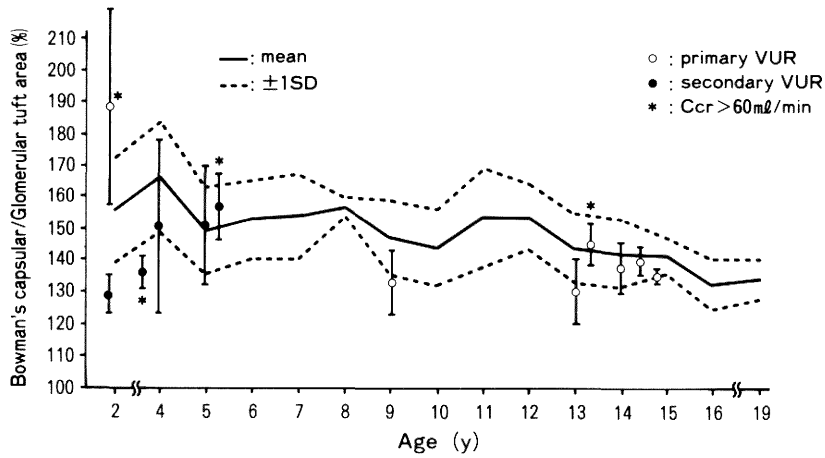

Fig. 11. Bowman's capsular/glomerular tuft area in children with reflux nephropathy.

drome (Table 4).

DISCUSSION

RN has been described as one of the definite causes of secondary FSGS[5]. When Cotran et al.[6] extensively re-evaluated kidney tissues obtained from 51 patients with VUR or obstructive pyelonephritis, 14 patients (28%) had FSGS lesions. On the other hand, there have been few pediatric reports of RN concerning FSGS in Japan, because the incidence of segmental sclerotic lesions is different and dependent on whether obtained kidney tissues contained the scarred area or not. Focal or segmental distribution of the lesions tended to be regarded as a minimal glomerular alteration, if serial sections were not extensively examined by light microscopy. We examined each glomerulus in serial sections to distinguish FSGS from minimal glomerular alteration (Fig. 14).

The hypothetical mechanism leading to FSGS in RN can be classified into two groups, immunological and nonimmunological processes. Immune complex- or cellular immunity-mediated glomerular injury have been suggested by the presence of glomerular

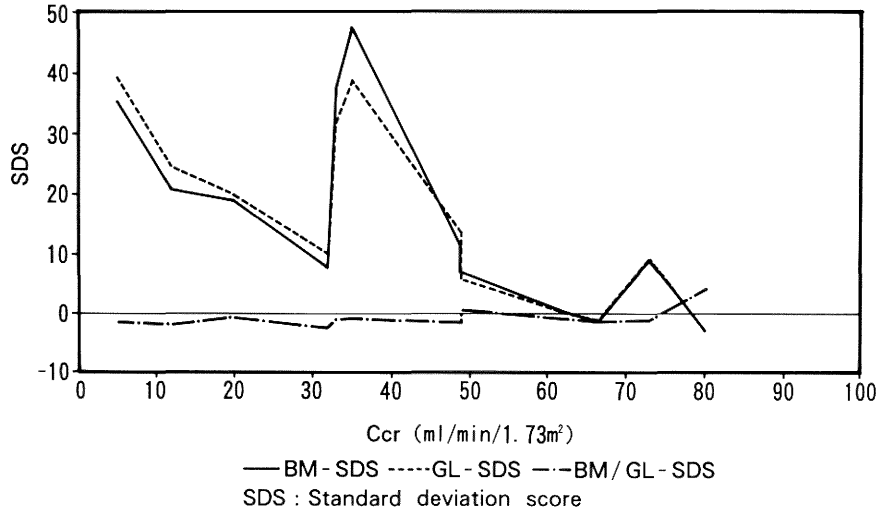

Fig. 12. Correlation between Ccr and SD score calculated by glomerular morphometric study in children with reflux nephropathy.

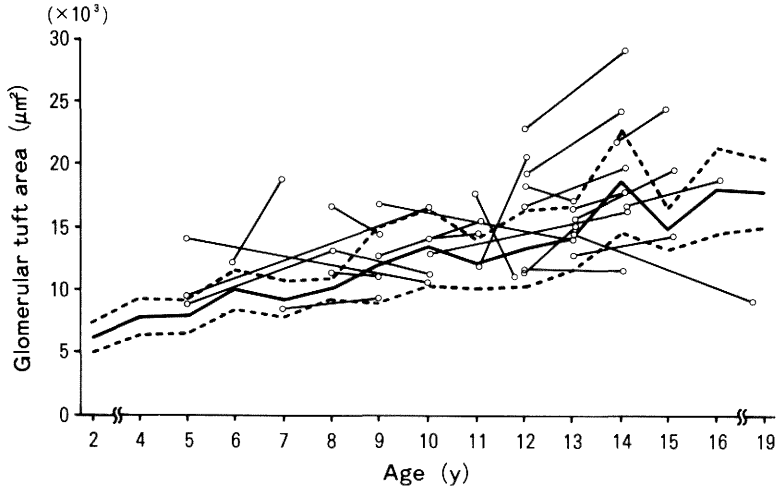

Fig. 13. Glomerular tuft area in serial biopsied children with IgA nephropathy.

deposition of immunoglobulins and complement components,[7] and extratubular localization of Tamm-Horsfall protein.[8] However, no glomerular deposition of Tamm-Horsfall protein was observed in this study. It was recently reported that an accumulation of extracellular matrix, activation of the complement system, and infiltration of suppressor/cytotoxic T cells and monocytes/macrophages were closely associated with glomerular obsolescence and the progression of RN.[9] Type III collagen in the obsolescent glomeruli of RN may be synthesized *in situ* or originated from the interstitium through synechiae because cell culture study has shown that mesangial cells produce Type III collagen[10] and Type III collagen was absent from normal mesangium. As it is possible that a primary disorder of the capillary tuft leads to nonspecific trapping of IgM and complement components in primary FSGS, pas-

Table 4. Comparison of glomerular morphometric parameters in primary FSGS and controls in glomerular morphometric study

		FSGS[1] (Ccr > 80 ml/m)	MCNS[2]	Hematuria[3] and MCNS	p-value 1) vs 2)	p-value 1) vs 3)
N		15	17	43		
Age (y)	:	11.7± 4.0	10.5± 3.6	11.1± 3.2	n.s.	n.s.
BM (μm^2)	:	23298.5±5085.8	18275.8±5310.6	19037.4±4237.5	<0.02	<0.01
GL (μm^2)	:	17050.4±3820.1	12611.8±3942.4	13077.2±3273.1	<0.01	<0.001
BM/GL (%)	:	137.1± 8.1	145.8± 6.0	147.3± 9.3	<0.002	<0.001

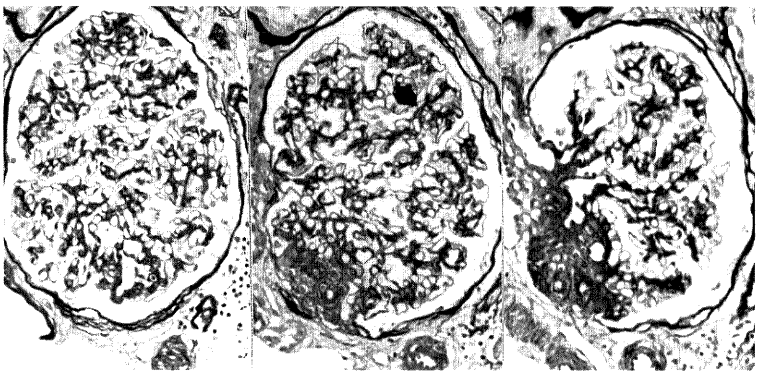

Fig. 14. The same glomerulus can be seen as minimal change or segmental sclerosis in a series of light microscopic sections with PAS stain.

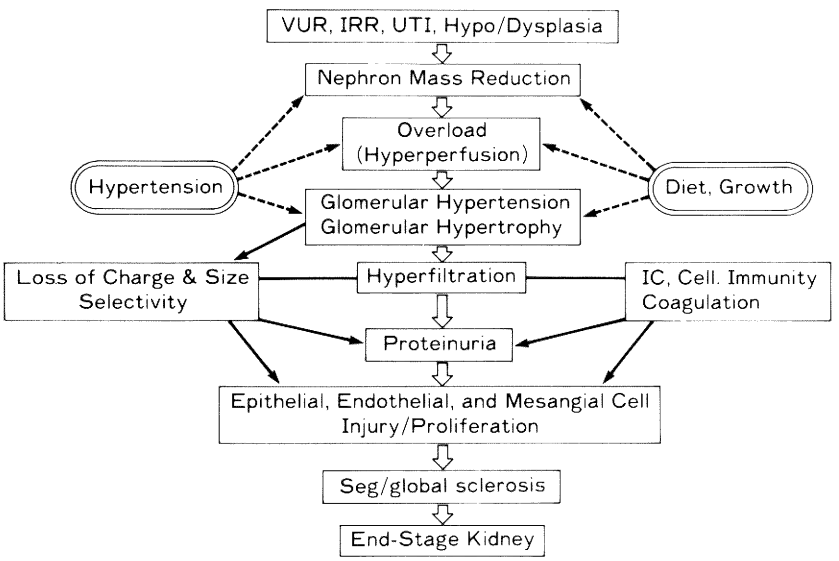

Fig. 15. Hypothetical mechanism of progression and exacerbation of reflux nephropathy.

sive glomerular deposition of those components in RN might also be proposed to mediate glomerular injury.

On the other hand, it has been supposed that a non-immunological process, such as a change in glomerular hemodynamics, rather than an immunological process, plays an important role in the formation of FSGS. There have been several reports focusing on the formation of FSGS: glomerular sclerosis in primary FSGS, which initially appeared in glomeruli located in the corticomedullary region[11] where the glomerular hemodynamic state was quite different from that in the outer cortical region; and glomerular overloading, hyperperfusion, hypertension or hyperfiltration has been shown in patients with unilateral renal agenesis[12] and oligomeganephronia,[13] and in five sixths nephrectomized rats.[14] It is of great interest that the present study proposed glomerular hyperperfusion or hypertension as possible causes of glomerular hypertrophy in RN, as well as in some cases of primary FSGS and IgA nephropathy. Glomerular injury associated with experimental persistent glomerular hypertension, induced by uninephrectomy plus deoxycorticosterone salt administration, was significantly ameliorated by normalization of systemic hypertension by antihypertensive treatment.[15] Glomerular hypertension, treated with a converting enzyme inhibitor or a low protein diet, stabilized an established glomerular injury.[16] Therefore, glomerular hypertension is likely an essential hemodynamic derangement responsible for progressive glomerular injury and normalization of glomerular hypertension may arrest the progression of remnant glomerular injury, even when therapy is started after glomerular injury is established. Moreover, it is meaningful for understanding the pathogenesis of FSGS in RN to remember that the location of segmental sclerosis in primary FSGS is closely correlated with the prognosis[17] and that primary FSGS was associated with glomerular hypertrophy, as compared with minimal change nephrotic syndrome[18]. Whereas IgA nephropathy is regarded as immune-complex-mediated glomerulonephritis, a nonimmunological process may influence the progress of this disease, as was the case with RN and primary FSGS. Conservative therapy, including good control of hypertension and placement on a low protein diet, should be reappraised in the treatment various renal diseases (Fig. 15). Very recently, it was suggested that because glomerular hypertrophy may not necessarily correlate to glomerular hypertension,[19,20] there may be a glomerular growth factor which induces glomerular hypertrophy in the absence of glomerular hypertension, and furthermore glomerular hypertrophy may be an important step preceding glomerular sclerosis,[21] In conclusion, when RN is diagnosed almost all patients have renal scarring and some of them also have hypoplastic/dysplastic lesions, and nephron mass reduction is definitely evident. Therefore, to arrest the deterioration to glomerular sclerosis, early management and therapy for RN are required. Since the high risk period of susceptibility to renal scarring due to VUR is now considered to be the ages of 4 to 5 years old, a medical administerial system for detecting urinary tract infection or VUR in early infancy should be established.

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