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Clinicopathologic Study of 20 Children with Reflux Nephropathy

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Summary. **To clarify the pathogenesis of reflux nephropathy (RN) leading to end-stage renal disease (ESRD), we investigated the clinicopathologic features of 20 children with RN and also compared urinary proteins in RN and primary focal glomerular sclerosis.**

The patients ranged from 3 months to 16 years of age at the time when kidney tissue was obtained, and included 15 boys and 5 girls. Imapired renal function (Ccr $<$ 70 ml/min 1.73 m^2) was observed in 10/12 patients with RN due to primary vesicoureteral reflux (VUR), and in 7/8 patients with RN due to secondary VUR. Light microscopy showed segmental glomerular sclerosis in 9/12 patients with RN due to primary VUR, and also moderate-to-severe tubulointerstitial changes. The onset of RN due to secondary VUR was earlier than that of RN due to primary VUR and the clinical outcome was worse. Most patients with secondary VUR and RN had more severe reflux and renal scarring than the primary VUR group. The main glomerular lesion in secondary VUR was globbal sclerosis, but tubuolointerstitial changes were similar to primary VUR.

There was significant glomerular enlargement and Bowman's capsule area enlargement in RN with a Ccr $<$ 70 ml/min 1.73 m^2. In 14 RN patients, a significant negative corrilation was found between Ccr and quantitative tubulointerstitial changes ($P < 0.01$). Proteinuria was chiefly albumin with a mixture of high and low molecular weight proteins.

Since nephron mass reduction was evident when RN was diagnosed in almost all patients, subsequent loss of renal function appears inevitable, mainly due to hemodynamic overloading. To arrest progression to ESRD, early detection and management of RN is required by establishing a screening system for detecting urinary tract infection or VUR.

INTRODUCTION

Reflux nephropathy (RN) is defined as renal parenchymal damage or scarring associated with vesicoureteric reflux (VUR),[1] and is one of the major causes of end-stage renal disease (ESRD) in children and young adults. The main cause of the progression of RN to ESRD despite aggressive medical or surgical treatment is thought to be remnant glomerular overload due to the loss of nephron mass that occurs with renal scarring. We have previously reported a close relationship between glomerular hypertrophy, probably resulting from glomerular hypertension, and segmental/global glomerular sclerosis in RN.[2] This sutdy was performed to clarify the pathogenesis of reflux nephropathy by comapring pathological findings, clinical features, and analysis of urinary proteins.

MATERIALS AND METHODS

Subjects

Kidney tissues obtained from 20 children with RN were examined in a histological and morphometric light microscopy study. The patients included 15 boys and 5 girls, and they were between 3 months and 16 years of age at the time of kidney biopsy or nephrectomy. There were 12 patients with primary VUR (a congenital condition resulting mainly from a structural deficiency of the vesicoureteric junction) and 8 patients with secondary VUR, which results from mechanical or functional obstruction in the lower urinary tract, e.g., due to posterior urethral valves or a neurogenic bladder.

To qualitatively compare proteinuria in RN, the urine and the kidney tissue were examined in 7 children with primary focal glomerulosclerosis (FGS) including 3 boys and 4 girls aged 4-15 years at the time of kidney biopsy. The endogenous creatinine clearance (Ccr) was between 31 and 128 ml/min/1.73 m^2 in these patients.

Hypertension was defined as a blood pressure greater than 140/90. Proteinuria was considered pres-

ent when a dipstick method showed 1+ or more.

Grading of VUR and scarring (Fig. 1)

VUR was graded according to the international classification system.[3] To morphologically grade RN by intravenous pyelography (IVP), Ikoma's pyelonephritis grading system,[4] a modification of Semllie's scarring grades, was used as follows: grade 0-no changes; grade I-deformity confined to the calyces or calyceal papillae; grade II- not more than two scarred aresa; grade III- more than three scarred areas with some normal parenchyma remaining; grade IV-generalized scarring; and grade V- end-stage shrunken kidney.

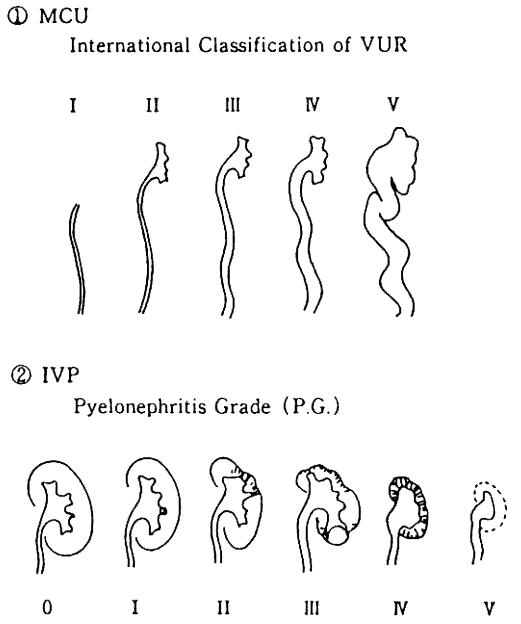


Fig. 1. International classification of VUR and Ikoma's pyelonephritis grade

Histomorphometric study

Serial histological sections (30 to 50) were prepared for qualitative and quantitative light microscopic studies. At least 3 glomeruli showing a cross-section at the glomerular equator were used for glomerular morphometry in each patient. An image analyzer (QTM720, Cambridge Corp., England or IBAS, Zeiss, Germany) was used for the morphometric study. Serial sections were stained by the periodic acid-Schiff (PAS) method. Quantitative measurement was performed to determine each Bowman's capsule area (BM area) and glomerular tuft area (GL area), and the Bowman's capsule area/glomerular tuft area ratio (BM/GL ratio, %) was also determined. The morphometric study was performed on glomeruli with minimal changes due to RN or primary FGS.

Two hundred and two glomeruli from 48 children (22 boys and 26 girls) with normal renal function (Ccr > 80 ml/min/1.73 m^2, minimal change nephrotic syndrome, or isolated microscopic hematuria (less than 30 red cells/field) served as the control glomeruli. The BM and GL areas, and the BM/GL ratio showed no statistical differences between the minimal change group and the isolated hematuria group.[2]

To allow for the effects of age on glomerular morphology and size, the Z score (standard deviation score, SDS), was calculated as follows: (mean − measured value)/appropriate SD for age.

The areas of tubular atrophy and interstitial fibrosis were measured quantitatively by image analysis and were expressed as the percentage change calculated as follows: [area of tubulointerstitial change × 100/area of the tubulointerstitial regions observed = T − I change (%)].

Semiquantitative grading of light microscopic changes

The number of glomeruli demonstrating segmental and global glomerular sclerosis were counted and scored as 0 to 3 according to the percentage of glomerular involvement (0% = no involvement; 1-20%; 1, 21-50%; 2, >50%; 3). The sum of the scores of segmental and global sclerosis comprised the sclerosis index.

Tubular atrophy and interstitial were also semiquantitatively graded from 0 to 3.

Qualitative investigation of urinary proteins

Urinary proteins were analyzed by three methods: 1) cellulose acetate electrophoresis (CAE), 2) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and 3) high-performance liquid chromatography (HPLC).

CAE was performed as described elsewhere.[5] SDS-PAGE was carried out using the Pharmacia Phast System with the Phast Gel Gradient 10-15. The urinary protein level was adjusted to 100 to 150 mg/dl by dilution with sample buffer or by concentration with a Minicon-B15 or Centricon-10 (W. R. Grace Co., Tokyo, Japan). HPLC was performed with the Pharmacia Fast Protein Liquid Chromatography system using a Mono Q anion-exchanger, as previously reported.[6] Five fractions were obtained as follows:

fraction I (0 to 5 min), fraction II (6 to 11 min), fraction III (12 to 17 min), fraction IV (18 to 23 min), and fraction V (24 to 29 min).

Urine samples were collected within 2 months before renal biopsy, centrifuged at 4℃, and stored at −20℃ until assayed.

Statistical analysis

All data are expressed as the mean±SD. Differences between groups were examined using the unpaired Student's t-test. Correlations were assessed using standard linear regression analysis by the least-squares technique. The results were considered statistically significant at $p<0.05$.

RESULTS

Clinicopathological features of RN (Tables 1-4)

Deterioration of renal function was defined as a Ccr less than 70 ml/min/1.73 m^2; it was observed in 10/12 children with RN due to primary VUR and in 7/8 children with RN due to secondary VUR at the time when kidney tissue samples were obtained. Four patients with RN due to primary VUR and 3 with RN due to secondary VUR progressed to ESRD at the final follow-up. Hypertension was present in 7 patients with RN due to primary VUR and in 2 with RN due to secondary VUR. Except for one patient with RN due to primary VUR and 3 patients with RN due to secondary VUR, all patients had proteinuria.

Table 1. Clinical profiles of the patients with reflux nephropathy due to primary VUR.

No.	Age (y)	Sex	Ccr (ml/m)	Prog-nosis	Hyper-tension	Prot-einuria	PG (r/l)	VUR (r/l)
1	14	m	5	HD	+	+	0/0(yhpo)	III/III
2	7	m	8	HD	+	+	IV/IV	IV/III
3	9	f	12	HD	+	+	0/0(hypo)	II/II
4	7	m	24	CRF	(−)	+	II/III	II/III
5	14	m	33	↓	+	+	II/IV	II/III
6	14	m	33	CRF	(−)	+	IV/III	V/V
7	14	m	35	HD	+	+	IV/IV	III/III
8	13	f	49	↓	(−)	+	III/III	0/1
9	16	f	65	→	+	+	II/II	II/II
10	15	m	66	→	(−)	+	III/II	III/IV
11	13	m	73	CRF	+	+	III/III	III/III
12	1	m	80	→	(−)	(−)	III/III	III/III

Ccr: creatinine clearance, PG: pyelonephritis grade, r: right, l: left, HD: hemodialysis, CRF: chronic renal failure, hypo: hypoplasia

Table 2. Clinical profiles of the patients with reflux nephropathy due to secondary VUR.

No.	Age (y)	Sex	Ccr (ml/m)	Prog-nosis	Hyper-tension	Prot-einuria	PG (r/l)	VUR (r/l)
1	8	m	5	HD	+	+	III/IV	IV/IV
2	6	f	8	HD	+	+	IV/agen	III/-
3	4	m	20	HD	(−)	+	IV/V	IV/IV
4	0	m	32	→	(−)	(−)	IV/IV	III/III
5	5	f	49	↓	(−)	±	IV/IV	IV/IV
6	2	m	62	acci. death	(−)	(−)	III/IV	I/III
7	5	m	67	↓	(−)	(−)	IV/IV	III/III
8	0	m	/	LFU	(−)	(−)	-/V	-/III

Ccr: creatinine clearance, PG: pyelonephritis grade, r: right, l: left, HD: hemodialysis, CRF: chronic renal failure, hypo: hypoplasia, LFU: lost to follow-up.

Table 3. Clinicopathologic findings in reflux nephropathy due to primary VUR.

No.	Age (y)	Sex	Ccr (ml/m)	Prog-nosis	No. of Glom	Mes Prol	Seg Scl	T-I change inf/fib & atr	Glom change	Others	
1	14	m	5	HD	64	+	+(h)	##	##/##	ESKidney	THP
2	7	m	8	HD	20	+	##(h.p)	##	##/##	ESKidney	
3	9	f	12	HD	5	0	##(h.p)	0	+/##	FSGS	
4	7	m	24	CRF	57	+	+(h.p)	##	##/##	ESKidney	
5	14	m	33	↓	3	0	0	0	0/0	minimal	
6	14	m	33	CRF	6	+	##(h)	0	+/##	FSGS	
7	14	m	35	HD	81	0	+(h.p)	##	##/##	ESKidney	THP dysplasia
8	13	f	49	↓ scar(−) scar(+)	2 8	0 0	0 +(h.p)	0 ##	+/0 +/##	minimal FSGS	
9	16	f	65	→	6	0	0	0	0/0	minimal	
10	15	m	66	→ scar(−)	6	+	+(h)	+	0/0	FSGS	
11	13	m	73	CRF CMJ scar(−) scar(+)	6 6 13	+ 0 0	##(h) 0 ##(h)	+ 0 +	##/## 0/0 ##/##	FSGS minimal FSGS	
12	1	m	80	→	23	+ (seg)	0	+	##/##	FGO	imm. gl

Glom: glomeruli, Mes Prol: mesangial proliferation, Seg Scl: segmental sclerosis, Glob Scl: global sclerosis, T-I: tubulointerstitial, inf/fib & atr: infiltration/fibrosis and atrophy, Glom: glomerular, HD: hemodialysis, CRF: chronic renal failure, CMJ: cortico medullary junction, h: hilar, p: peripheral, ESKidney: end-stage kidney, FSGS: focal segmental glomerulosclerosis, FGO: focal global obsolescence, THP: Tamm-Horsfall protein, imm gl: immature glomeruli.

Table 4. Clinicopathologic findings in reflux nephropathy dur to secondary VUR.

No.	Age (y)	Sex	Ccr (ml/l)	Prog-nosis	No. of Glom	Mes Prol	Seg SCl	T-I change inf/fib & atr	Glom change	Others	
1	8	m	5	HD	10	0	0	##	##/##	ESKidney	THP
2	6	f	8	HD	26	0	0	+	+/+	FGO	
3	4	m	20	HD	4	+ (seg)	0	+	##/##	FGO	
4	0	m	32	→	18	+ (seg)	0	##	##/##	FGO	imm.gl
5	5	f	49	↓	15	+ (seg)	0	##	##/##	FGO	
6	2	m	62	acci. death	65	0	0	0	0/±	minimal	
7	5	m	67	↓ scar(−)	41	0	0	0	0/0	minimal	
8	0	m	/	LOF	7	0	0	0	0/±	minimal	dysplasis

For abbreviations, see the legend to Table 3.

Most of the patients with RN due to either type of VUR had more than grade III reflux and renal scarring.

Light microscopy showed segmental glomerular sclerosis in 9/12 patients with RN due to primary VUR, and moderate-to-severe tubulointerstitial changes in 9 patients. In the unscarred area of the biopsy specimens obtained from patients No. 8 and 11, the glomerular lesions were minimal at the light microscopic level on the 50 serial sections examined. However, electron microscopy of glomeruli obtained from unscarred areas demonstrated epithelial cell changes and subendothelial electron-dense deposits (Fig. 2). In the scarred regions and cortico-medullary zones, segmental glomerular sclerosis was observed in 5 patients (6 specimens). This segmental sclerosis was mainly located near the vascular poles in 4 patients (5 specimens), and at both the vascular pole and in the peripheral glomerular tufts in 5 patients (5 specimens). These areas of segmental sclerosis were negative for colloidal iron staining, suggesting a reduction in sialic acid content. Striated PAS- positive material was found in the tubular lumens or interstitial regions in 3 patients, suggesting the presence of Tamm-Horsfall protein. Interstitial foam cells were observed in 2 patients.

In comparison with RN due to primary VUR, the age of onset of RN due to secondary VUR was lower and the clinical outcome was worse. Most patients with RN due to secondary VUR had more severe

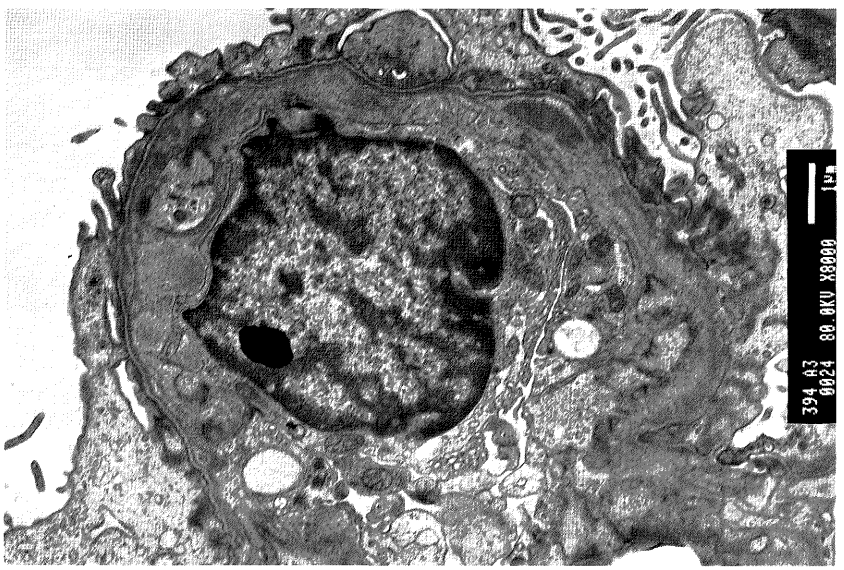


Fig. 2. Electron microscopic findings of glomeruli obtained from unscarred area showed alterations of epithelial cells and GBM, and subendothelilal electrondense deposits (×8000).

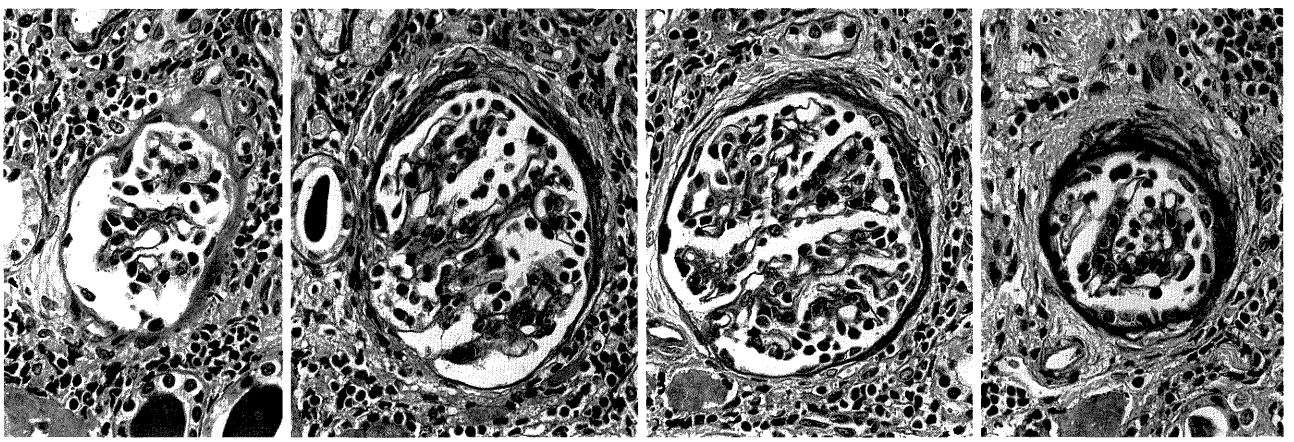


Fig. 3. Atubular glomerulus, disconecting to proxymal tubule, can be seen in serially sectioned specimens (PAS ×200)

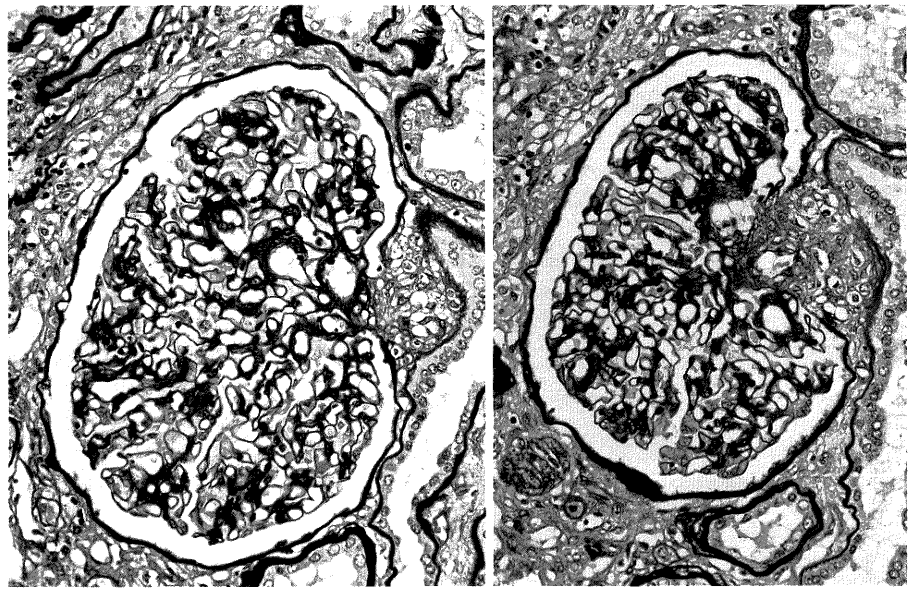


Fig. 4. Glomerulus accompanied with hyperplasia of extraglomerular mesangium can be seen (PAS ×200).

reflux and renal scarring than the patients with nephropathy due to primary VUR. The glomerular change in secondary VUR was mainly global sclerosis rather than segmental sclerosis, while the tubulointerstitial changes were similar to those in the primary VUR group. Other findings included immature glomeruli and dysplasia. Atubular glomeruli were observed in two patients (Fig. 3). Glomeruli with hyperplasia of the extraglomerular mesangium were seen more frequently than is usual in various types of chronic glomerulonephritis (Fig. 4).

Although three patients (case M.K., S.H., and N.H.) were normotensive, they were treated with the ACE inhibitor, captopril (10-15 mg/day) and a low protein diet (0.8-1.0 g/kg/day) during the follow-up period (range 10 months to 2.5 years).

Histomorphometric study (Tables 5 and 6)

There was a significant increase of the BM and GL areas in RN associated with a decrease in Ccr to less than 70 ml/min/1.73 m^2. This glomerular hypertrophy was independent of the presence of scarring. Although glomerular hypertrophy was evident in glomeruli exhibiting minimal-to-mild proliferative changes, it was not apparent in glomeruli with moderate-to-severe proliferation, synechiae, or global sclerosis. There was a significant negative correlation between Ccr and the GL or BM area SDS in various glomerulonephritides ($p<0.01$), and a rapid increase in the SDS of both areas when the Ccr diminished to under 60-70 ml/min/1.73 m^2 (Fig. 5-a).

In 14 patients with RN, a sighificant negative correlation was found between Ccr and the quantitatively evaluated tubulointerstitial changes (T-I change %, $P<0.01$, Fig. 5-b).

Qualitative analysis of urinary proteins

1) *CAE* (Table 7)

The gamma globulin fraction (% gamma) tended to increase in association with a decrease in Ccr, while the beta globulin fraction tended to decrease along with the Ccr. The albumin/gamma globulin ratio was significantly higher in RN than in primary FGS.

2) *SDS-PAGE* (Table 8)

In association with progressive impairment of renal function and glomerulo-tubular damage, the urinary albumin fraction (MW 68,000) decreased, while the low molecular weight fraction (MW <40,000) increased. The urinary albumin and low molecular weight fractions both showed significant differences between RN and primary FGS (77.1 ± 9.6 versus 86.5 ± 3.8, 17.6 ± 7.3 versus 7.2 ± 4.4, both $p<0.05$). The urinary albumin/low molecular weight protein rotio also showed a significant difference between RN and primary FGS (3.3 ± 2.2 versus 14.9 ± 5.3, $p<0.01$).

3) *HPLC* (Table 9)

The Value of urinary fraction II in RN, including beta 2 microglobulin, was significantly higher than

Table 5. Morphometric study of reflux nephropathy due to primary VUR.

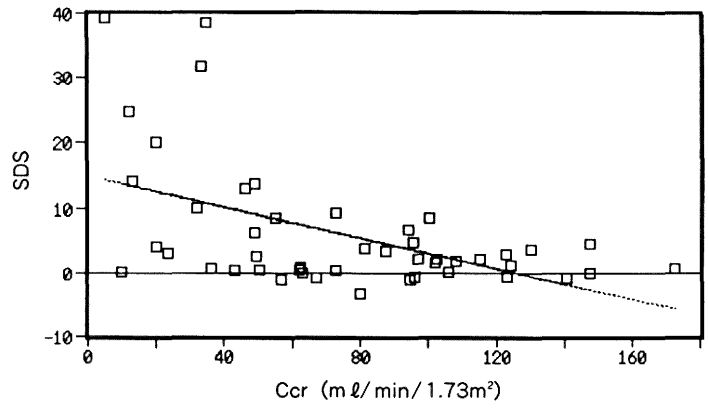
No.	Age (y)	Sex	Ccr (ml/m)	Prognosis	GL-SDS	BM-SDS	BM/GL-SDS	T-I (%)
1	14	m	5	HD	9	9	0	55
2	7	m	8	HD	17	13	−2	54
3	9	f	12	HD	8	7	−1	31
4	7	m	24	CRF	2	2	0	52
5	14	m	33	↓	8	8	−1	8
6	14	m	33	CRF	8	8	−1	28
7	14	m	35	HD	10	10	0	59
8	13	f	49	↓	17	17	0	4
				scar(−)	16	14	−1	33
				scar(+)				
9	16	f	65	→	3	5	3	16
10	15	m	66	→	9	19	−1	9
				scar(−)				
11	13	m	73	CRF	18	15	−2	29
				CMJ	17	15	−1	4
				scar(−)	16	17	0	32
				scar(+)				
12	1	m	80	→	0	0	2	70

GL-SDS: glomerular standard deviation score, BM: Bowman, T-I(%): tubulointerstitial change(%), LFU: lost to follow-up

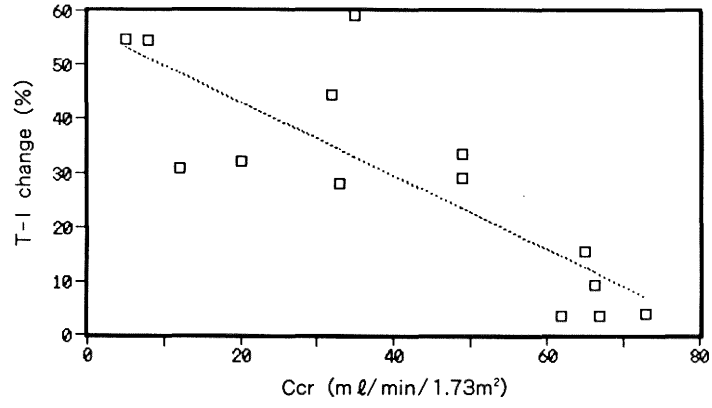
Table 6. Morphometric study of reflux nephropathy due to secondary VUR.

No	Age (y)	Sex	Ccr (ml/m)	Prognosis	GL-SDS	BM-SDS	BM/GL-SDS	T-I (%)
1	8	m	5	HD				
2	6	f	8	HD				
3	4	m	20	HD	17	16	−1	32
4	0	m	32	→	13	10	−2	44
5	5	f	49	↓	17	23	0	29
6	2	m	62	acci. death	7	6	−1	3
7	5	m	67	↓	6	6	−1	4
				scar(−)				
8	0	m	/	LFU				

Gl-SDS: glomerular standard deviation score, BM: Bowman, T-I (%): tubulointerstitial change (%), LFU: lost to follow-up

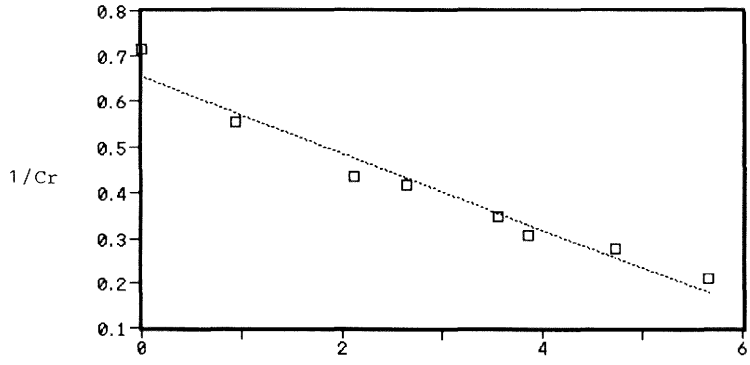


(a)

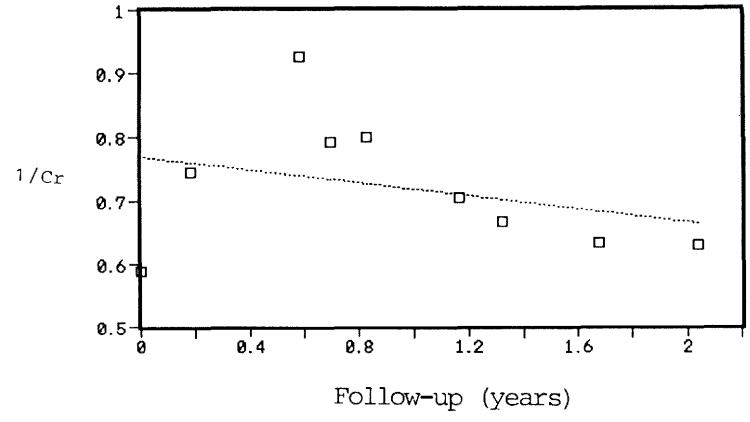


(b)

Fig. 5. (a): Correlation between Ccr and GL-SDS in various glomerulonephritides ($N=47$, $r=0.505956$, $y=-0.119201x+14.977180$, $p<0.01$)(b): Correlation between Ccr and quantitatively evaluated tubulointerstitial change in reflux nephropathy ($N=14$, $r=-0.823366$, $y=-0.678703x+56.628356$, $p<0.01$).



(a)



(b)

Fig. 6. The slope of the regression line of the reciprocal serum creatinine versus time. (a): a patient with no treatment ($y=-0.083876x+0.6547537$). (b): a patient with a low protein diet (1 g/kg/day) and Captoprill (15 mg/day) ($y=-0.051418x+0.7693116$).

Table 7. Cellulose acetate electrophoresis of proteinuria in reflux nephropathy.

Age	Sex	Alb (%)	α1 (%)	α2 (%)	α1+2 (%)	β (%)	γ (%)	A/G	Alb/γ	Ccr (ml/m)	Glomer Change	Scler Index	T-I Change	T-I (%)	Glomer SDS
16	f	74.0	3.0	4.5	7.5	16.8	2.1	2.8	26.4	66	minimal	0	(−)	16	2.6
13	m	78.7	3.1	2.9	6.0	10.3	4.9	3.7	21.2	59	seg>glob	3	++	30	16.2
7	m	70.3			12.1	7.5	10.0	2.4	29.6	24	seg<glob	4	+++	52	2.9
9	f	75.3	3.6	5.0	8.6	7.7	8.3	3.1	24.7	11	seg>glob	2	+++	31	8.4
7	m	77.1	1.4	2.7	4.1	6.8	12.0	3.4	22.9	8	seg<glob	4	+++	54	16.9
11	f	74.4	6.9	4.3	11.1	7.2	7.2	2.9	25.6	5	glob	2	++	28	6.9
14	m	69.5			9.2	15.2	6.1	2.3	30.5	5	seg<glob	4	+++	55	9.1
RN(N=7)															
	M	74.2	5.6	3.9	8.4	10.2	7.3	2.9	25.8						
	SD	3.1	3.6	0.9	2.6	3.8	3.1	0.5	3.1						
FGS(N=6)															
	M	72.0	7.6	5.4	11.2	11.3	5.1	3.3	15.8						
	SD	11.1	2.8	4.1	6.3	7.4	1.8	1.9	5.5						
RN vs FGS															
	P	NS	NS	NS	NS	NS	NS	NS	<0.01						

Ccr: creatinine clearance, Seg Glob:segmental, global sclerosis, Scler: glomerular sclerosis, T-I: tubulointerstitial, Glomer: glomerular, SDS: standard deviation score, Alb: slbumin, A/G: albumin/globulin, M: mean, SD: standard deviation.

Table 8. SDS-polyacrylamide gel electrophoresis of proteinuria in reflux nephropathy.

Age	Sex	>7.0 (%)	6.8 (%)	≧6.8	6.7-4.1	≧4.0	≦4.0	Ccr (ml/m)	Glomer Change	Scler Index	T-I Change	T-I (%)	Glomer SDS
15	m	8.2	78.7	86.9	3.5	9.5	8.3	66	minimal	0	(−)	9	8.5
16	f	54.6	32.7	87.3	0.2	12.6	2.6	66	minimal	0	(−)	16	2.6
13	m			90.0	3.9	6.0		59	seg>glob	3	++	30	16.2
14	m			75.6	3.2	21.2		33	seg>glob	3	++	28	8.0
7	m			66.3	4.9	28.3		24	seg<glob	4	+++	52	2.9
4	m	23.7	44.7	68.4	10.8	20.8	2.1	20	glob	2	+++	32	3.1
9	f	23.7	44.7	68.4	10.8	20.8	2.1	11	seg>glob	2	+++	31	8.4
7	m	23.3	47.1	70.4	7.1	22.5	2.1	8	seg<glob	4	+++	54	16.9
11	f	24.1	43.7	67.8	1.2	25.5	1.7	5	glob	2	++	28	6.9
14	m	54.0	35.8	89.8	1.3	8.9	4.0	5	seg<glob	4	+++	55	9.1
RN(N=10)													
	M	30.2	46.8	77.1	4.7	17.6	3.3						
	SD	16.1	13.9	9.6	3.6	7.3	2.2						
FGS(N=6)													
	M	26.8	62.0	86.5	6.3	7.2	14.9						
	SD	3.8	2.7	3.8	1.7	4.4	5.3						
RN vs FGS													
	P	NS	<0.05	<0.05	NS	<0.05	<0.01						

For abbreviations, see legend to Table 7.

Table 9. High-performance liquid chromatography of proteinuria in reflux nephropathy.

Age	Sex	Fraction No.					α1-AG	TF	RBP	V/I	V/α1AG	I/TF	V/RBP	Ccr (ml/m)	Glomer change	Scler Index	T-I change	T-I (%)	Glomer SDS
	(y)	I	II	III	IV	V													
8	m	9.6	21.8	15.4	27.3	24.1	27.0	6.0	4.6	2.5	0.9	1.6	5.3	24	seg<glob	4	+++	52	2.9
14	m	12.1	16.5	20.2	21.5	29.6	21.5	5.5	4.5	2.5	1.4	2.2	6.6	5	seg<glob	4	+++	55	9.1
11	f	4.7	17.8	21.9	25.5	21.8	23.5	5.4	9.5	4.6	0.9	0.9	2.3	5	glob	2	++	28	6.9
10	f	14.3	16.3	16.4	11.8	33.2	11.8	5.3	4.7	2.3	2.8	2.7	7.0	11	seg>glob	2	+++	31	8.4
15	m	12.9	12.5	20.4	9.6	35.0	9.6	4.1	8.3	2.7	3.7	3.1	4.2	66	minimal	0	(-)	9	8.5
16	f	13.8	7.3	23.3	19.7	31.2	19.7			2.3	1.6			66	minimal	0	(-)	16	2.6
8	m	3.2	12.3	22.0	19.4	41.3	19.4			12.8	2.1			8	seg<glob	4	+++	54	16.9
5	m	8.9	16.7	21.8	19.9	12.6	19.9			1.4	0.6			20	glob	2	++	32	3.1
13	m	22.7	8.6	21.1	8.3	38.9				1.7				59	seg>glob	3	++	30	16.2
RN(N=9)																			
	M	11.4	14.4	20.3	18.1	29.8	19.0	5.3	6.3	3.7	1.8	2.1	5.1						
	SD	5.4	4.4	2.5	6.4	8.5	5.4	0.6	2.1	3.4	1.0	0.8	1.7						
FGS(N=7)																			
	M	7.1	8.2	17.8	13.4	50.9	14.6	4.3	6.7	11.3	3.5	3.1	8.7						
	SD	4.4	3.7	6.4	5.5	13.6	4.7	1.7	3.3	10.2	1.1	3.6	4.0						
RN vs FGS																			
		p	<0.05	NS	NS	<0.01	NS	NS	NS	<0.1	<0.05	NS	NS						
		NS																	

For other abbreviations, see legend to Table 7.

that in primary FGS (14.4±4.4 versus 8.2±3.7, $p<0.05$), whereas the value of fraction V, albumin fraction, in RN was significantly lower than that in primary FGS (29.8±8.5 versus 50.9±13.6, $p<0.01$). The urinary fraction ratio of V/alphal acid glycoprotein showed a significant difference between RN and primary FGS (1.8±1.0 versus 3.5±1.1, $p<0.05$). Changes in renal function following treatment with a low protein diet and captopril (Fig. 6)

When the slope of the regression line for the reciprocal of serum creatinine versus time (1/Cr) was compared between group A (3 patients with no treatment) and group B (3 patients on a lowprotein diet and captopril), group B showed a significantly lower value for the mean slope than group A (−0.088466± 0.003641 versus −0.061072±0.007153).

DISCUSSION

The incidence of ESRD in children with RN has been reported to be 0.3-0.4 per million per year, or 7.1-34%.[7,8] However, it is possible that some children with severe RN who progress to ESRD in early infancy have been overlooked in these statistics. Since RN contributes much to ESRD in the young, research into its pathogenesis is needed to allow us to identify such patients as soon as possible.

Although the clinical factors involved in determining the patients in whom progression will occur have not been thoroughly established, several reports have shown the following prognostic indicators for the deterioration of renal function: (1) impaired renal function at presentation; (2) proteinuria of 2+ or greater on spot urinalysis or greater than 1 g/day; (3) hypertension; and (4) short stature of below the fifth percentile.[9] Most of our patients in the present series with a poor prognosis had 2 or more of these indicators.

Though RN has been described as one of the definite causes of secondary focal segmental glomerulosclerosis (FSGS), there have been few pediatric reports of RN in relation to FSGS in Japan. This may be explained by differences in the incidence of segmental sclerotic lesions and by dependence on whether the biopsied kidney tissues contains a scarred area or not, as well as by the focal or segmental distribution of the lesions leading them to be regarded as minimal glomerular alterations. Since atubular glomeruli may constitute a larger percentage of the glomeruli in chronic pyelonephritis with little or no sclerosis than was previously thought,[10] it is likely that tubulointerstitial changes rather than glomerular changes were closely correlated with the deterioration of renal function noted in the present study.

The potential mechanisms leading to FSGS in RN

can be classified into two general groups, which include immunological and nonimmunological processes. Because of the detection of glomerular deposition of immunoglobulins and complement components[11] and the extratubular localization of Tamm-Horsfall protein,[12] the role of immune complex- and/or cellular immune-mediated glomerular injury has been suggested. However, no glomerular deposition of Tamm-Horsfall protein was observed in this study. It was recently reported that accumulation of the extracellular matrix, activation of the complement system, and infiltration of suppressor/cytotoxic T cells and monocyte/macrophages were closely associated with glomerular obsolescence and the progression of RN.[13] As it is possible that a primary disorder of the capillary tuft leads to the nonspecific trapping of IgM and complement components in primary FSGS, passive glomerular deposition of these materials might also be proposed to mediate glomerular injury in RN.

On the other hand, it has been suggested that a nonimmunological process, including changes in glomerular hemodynamics and growth factors, may play an important role in FSGS. Several reports account for the formation of FSGS by a derangement of glomerular hemodynamics. For example, glomerular sclerosis in primary FSGS may initially appear in the glomeruli of the corticomedullary region[14] where glomerular hemodynamics are quite different from the outer cortical region. In addition, glomerular overloading, hyperperfusion, hypertension, and hyperfiltration have all been observed in patients with unilateral renal agenesis[15] and oligomegaenephronia,[16] and in five-sixths nephrectomized rats.[17]

It has recently been suggested that there may be a glomerular gorwth factor which induces hypertrophy in the absence of glomerular hypertension, and that glomerular hypertrophy may be an important step preceding glomerular sclerosis,[18] because such hypertrophy does not necessarily appear to be correlated with glomeruslar hypertension.[19,20] It is of great interest that the present study found glomerular hypertrophy in RN patients, although no exact cause could be identified.

Concerning therapy, experimental persistent glomerular hypertension induced by unilateral nephrectomy plus desoxycorticosterone-salt administration, is associated with significantly less glomerular injury after the normalization of systemic hypertension by antihypertensive therapy.[21] Treatment of glomerular hypertension with an ACE-inhibitor or a low protein diet has also been shown to stabilize established glomerular injury.[22] In contrast to the excellent effect of ACE-inhibitors in animal experiments, their efficacy in humans with chronic renal disease has been controversial. In this regard, delayed therapy and low ACE-inhibitor doses may contribute to the variable results, because early therapy at higher doses produces positive results in animals with subtotal nephrectomy.[23]

Since glomerular hypertension is likely to be an essential hemodynamic derangement responsible for progressive glomerular injury, normalization of this hypertension could possibly arrest the progression of remnant glomerular injury, even when therapy has been delayed until glomerular injury is already established. Moreover, since it has been reported that angiotensin II causes the hypertrophy and of mesangial cells and an increase in matrix production without any concomitant hyperplasia,[24] the present methods of preliminary conservative therapy (including good control of hypertension and a low protein diet) should be reappraised in the management of RN and various other chronic renal diseases.

There are a few reports analyzing proteinuria and renal histologic changes in RN. Torres et al.[25] stated that all patients with ESRD due to RN had significant proteinuria (0.5-10.4 g/day, 2.8 ± 0.3 g/day) at the time of their initial evaluation and that urinary protein electrophoresis showed that albumin accounted for most of the proteinuria ($62 \pm 6\%$). Boesken[26] found tubular proteinuria with additional excretion of transferrin and IgG in patients with chronic pyelonephritis or interstitial nephritis (some of them verified by histology) using SDS-PAGE. The results of our analysis of proteinuria in patients with RN complicated by glomerular and tubulointerstitial changes were almost the same as Boesken's. Therefore, proteinuria due to RN might be composed chiefly of albumin in association with mixture of high and low molecular weight proteins, depending on the extent of glomerulotubular damage. This may suggest some measures for the early detection of RN before irreversible changes have occurred.

In conclusion, almost all of our patients had renal scarring and some of them also had hypoplastic/dysplastic lesions. Nephron mass reduction was definitely evident at the time when RN was diagnosed. Therefore, to arrest the deterioration to glomerular sclerosis, the early detection and management of RN is probably required. Since the high risk period for susceptibility to renal scarring due to VUR is up to 4 or 5 years of age, a system should be established to detect UTI or VUR by urinalysis (for leukocyturia and low molecular weight protein) and to allow the ultrasonic diagnosis of renal scarring or

hypoplasia in early infancy.

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