Original Article

Synthetic/Secreting and Apoptotic Phenotypes in Renal Biopsy Tissues from Hypertensive Nephrosclerosis Patients

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The major glomerular abnormalities in hypertensive nephrosclerosis are described as glomerular obsolescence (GO), glomerulosclerosis (GS), and glomerular collapse (GC). However, glomerular cellular changes caused by hypertensive insults have not been well analyzed. Using an immunoenzyme method, we examined eleven biopsy samples from patients with hypertensive nephrosclerosis for two synthetic and secreting phenotypes, α-smooth muscle actin (α-SMA) and collagen type III (Col. III), and two apoptotic phenotypes, pro-apoptotic molecule Bax and anti-apoptotic molecule Bcl-2. Together with the glomerular and vascular changes and interstitial fibrosis (IF) area, the results were scored quantitatively and semi-quantitatively and compared to the clinical findings, which included systolic blood pressure (SBP), mean arterial pressure (MAP), serum creatinine levels (sCr) and creatinine clearance (Ccr), using univariate and multivariate analyses. As a result, GS was frequently observed in the mild-to-moderate hypertensive group (140≤SBP<180 mmHg), whereas GC was positively correlated with SBP. Furthermore, there was a positive correlation of GS with mesangial α-SMA and Col. III, suggesting that GS was the reflection of these synthetic and secreting phenotypic changes in mesangial cells. Endothelial Bax was positively correlated with Ccr ($p<0.01$); in contrast, podocytic Bax was positively correlated with sCr ($p<0.05$) and showed a tendency to correlate with MAP ($p=0.054$). In conclusion, these findings support the view that mesangial synthetic and secreting phenotypic changes may be a reflection of cellular activation caused by mild-to-moderate hypertension and that apoptotic phenotypic expression in podocytes, rather than endothelial cells, may be related to the development of a severe form of hypertensive nephrosclerosis. (*Hypertens Res* 2006; 29: 573–580)

***Key Words*: α-smooth muscle actin, collagen type III, Bax, Bcl-2**

Introduction

Hypertension-induced nephrosclerosis is frequently the major cause of end-stage renal disease. The primary lesions are vascular lesions such as hyalinization and medial wall thickening in benign nephrosclerosis and onion-skin lesions or fibrinoid necrosis in malignant nephrosclerosis. Furthermore, glomerular damages are the major pathological events leading to renal fibrosis. Two distinct glomerular lesions, glomerulosclerosis (GS) and glomerular collapse (GC), which are recognized in the glomeruli, may potentially lead to complete glomerular obsolescence (GO). In benign nephrosclerosis, segmental GS and complete GO are the characteristic pathologic findings. In malignant nephrosclerosis, the most frequently seen glomerular lesions are capillary fibrinoid necroses. Furthermore, a high frequency of ischemic GC with garland-like wrinkling of the basement membranes has been described as a major find-

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Table 1. Clinical Profile of the Patients

	Age/sex	SBP/DBP (mmHg)	MAP (mmHg)	Tx	sCr (mg/dl)	Ccr (ml/min)	PTN (g/day)	No. of glo	A. sclerosis*	A. necrosis	GS	GO (%)	GC (%)	IF (%)
1	77/f	142/90	107.3	+	1.1	40	1.2	15	11/13	+	2.3	27	0	90.3
2	77/m	144/70	94.7	+	1.5	27	3.9	10	10/19	–	3.2	50	8	99.6
3	68/m	156/52	86.7	–	1.5	51	2.8	10	4/6	–	1.6	20	20	88.2
4	60/f	160/80	106.7	+	0.9	107	0.1	10	5/8	–	2.2	40	0	89.2
5	64/f	160/90	113.3	+	0.9	52	0.2	13	4/4	–	2.5	50	4	71.8
6	39/m	162/112	128.7	–	3.3	23	1.2	22	26/26	+	1.5	28	25	95.7
7	60/f	180/100	126.7	+	2.5	16	0.9	11	30/30	+	2.3	41	29	98.9
8	61/f	180/98	125.3	+	0.6	100	0.1	12	14/14	–	0.6	14	7	69.1
9	31/m	190/148	162.0	+	2.4	28	3.8	12	4/7	–	2.5	58	16	92.5
10	23/m	192/100	130.7	+	0.9	92	0.4	10	7/10	–	1.2	20	20	57.6
11	32/f	220/130	160.0	+	2.3	28	3.5	18	3/3	+	0.8	19	57	99.3
Mean		171/97	122.0		1.6	51.3	1.6	13	10.7/12.7		1.9	33.4	16.9	86.6

*Positive number per total number; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; Tx, treatments with antihypertensive drugs; sCr, serum creatinine level; Ccr, creatinine clearance; PTN, urinary protein; No. of glo, number of glomeruli obtained; A. sclerosis, arterio-arteriolosclerosis; A. necrosis, arterio-arteriolar necrosis including onion-skin lesion and fibrinoid necrosis; GS, glomerulosclerosis; GC, glomerular collapse; GO, glomerular obsolescence; IF, interstitial fibrosis; m, male; f, female.

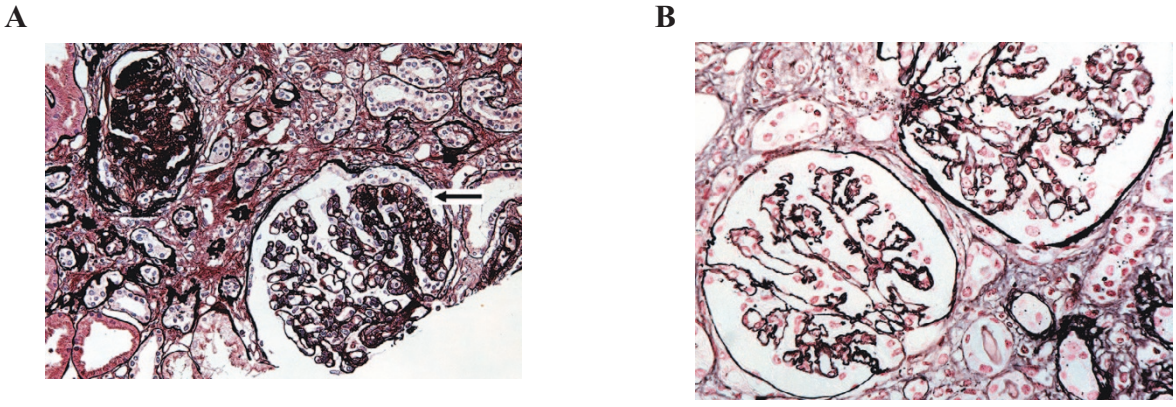

Fig. 1. *Characteristic glomerular findings in nephrosclerosis (periodic acid–methenamine staining). A: Glomerulus at the right bottom of the image shows segmental glomerulosclerosis (arrow). Obsolescent glomerulus is shown at the top left of the image. B: Glomerular collapse with garland-like wrinkling.*

ing in nephrosclerosis, and is more frequently observed in malignant than benign nephrosclerosis (*1*, *2*).

With respect to the mechanism of progression, an enhanced intrarenal effect of angiotensin II and enhanced transforming growth factor β-1 (TGFβ-1) production due to endothelial cell impairment appear to contribute to hypertensive nephrosclerosis (*3*). In addition, recent reports have shown that a reduction of nitric oxide (NO) may contribute to the development of hypertensive nephrosclerosis (*4*, *5*). From the perspective of hemodynamics, hypertensive shear stress and stretch forces are assumed to stimulate glomerular cells to differentiate with the new acquisition of synthetic and secreting phenotypes (*6*). Furthermore, severe hypertensive damage may cause apoptosis of glomerular cells (*7–10*). Ying *et al*. (*11*) and Ying and Sanders (*12*) observed that an apoptotic event was increased in salt-sensitive rats on a high-salt diet, causing irreversible hypertensive renal damage, and suggested that the mitochondrial pathway was strongly involved.

In the present study, we focused on both synthetic/secreting phenotypes and apoptosis-related phenotypes. Using human kidney samples, we immunohistologically examined the glomerular expressions of α-smooth muscle actin (α-SMA) and collagen type III (Col. III) as respective markers of the synthetic and secreting phenotypes, of Bax as a marker of apoptosis acceleration, and of Bcl-2 as a marker of apoptosis inhibition. The results were compared with various clinicopathological findings.

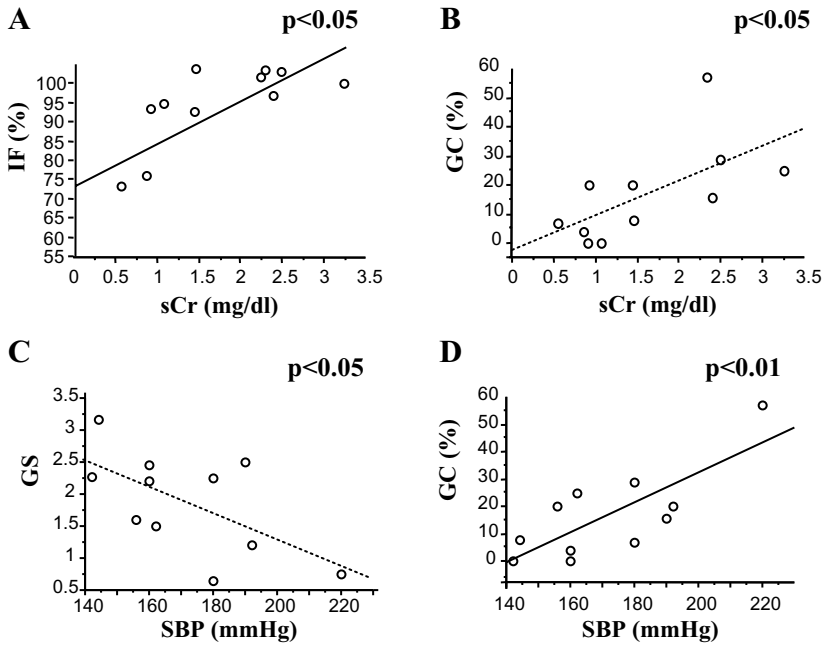

Fig. 2. *Relationships of sCr with IF (A) and GC (B). There were positive correlations of sCr with GC and IF, but after adjusting for age and proteinuria, IF arose as the independent factor which contributed to the increased sCr. Relationships of SBP with GS (C) and GC (D). There was a negative correlation between GS and SBP by simple linear correlation, but not by multivariate analysis including age and proteinuria as the parameters. In contrast, SBP was positively correlated with GC.*

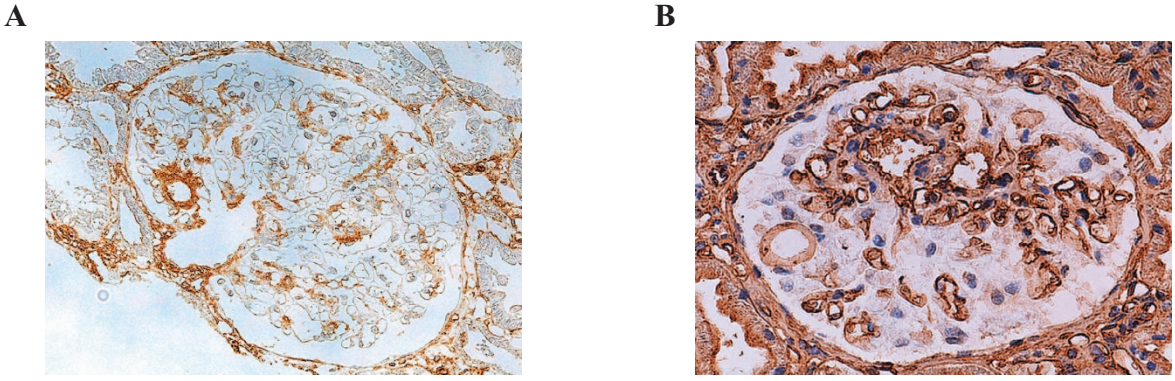

Fig. 3. *Immunoenzyme staining of Mes α-SMA (A) and Col. III (B). Both panels show diffuse mesangial staining of grade 3.*

Methods

Patient Selection

Ten Japanese patients admitted to the Medical Research Institute of Kitano Hospital and one patient admitted to Himeji National Hospital underwent renal biopsy for the examination of hypertension and various degrees of proteinuria. On light microscopy and fluorescent antibody test, diabetic nephropathy and primary glomerular diseases such as nephrotic syndrome or nephritis were excluded, and a diagnosis of hypertensive nephrosclerosis was made. Only one patient (case 2) was examined by electron microscopy. All the cases were evaluated based on the severity of hypertension, proteinuria, and two indices of renal function, *i.e.*, serum creatinine level (sCr) and creatinine clearance (Ccr). There were 5 males and 6 females, and their patient data were as follows (mean±SD): age, 54±19 years; systolic/diastolic blood pressure (SBP/DBP), 171±23/97±27 mmHg; mean arterial pressure (MAP), 122±24 mmHg; sCr, 1.6±0.9 mg/dl; Ccr, 51±33 ml/min; urinary protein (PTN), 1.6±1.5 g/day. Before new treatments were started, supine blood pressure measurements were obtained in the morning between 7 AM and 8 AM

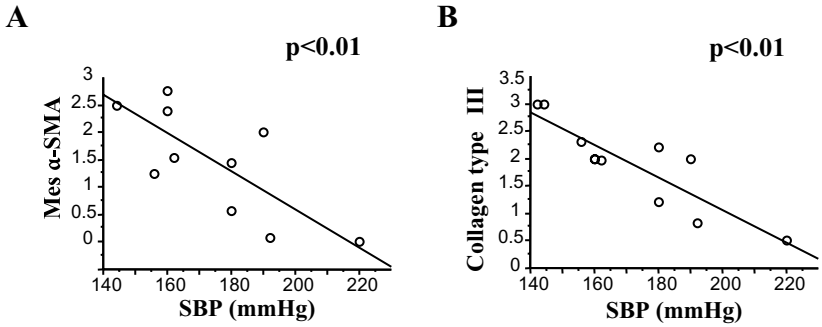

Fig. 4. *Relationships of Mes α-SMA (A) and Col. III (B) with SBP. Both Mes α-SMA and Col. III were inversely correlated with SBP.*

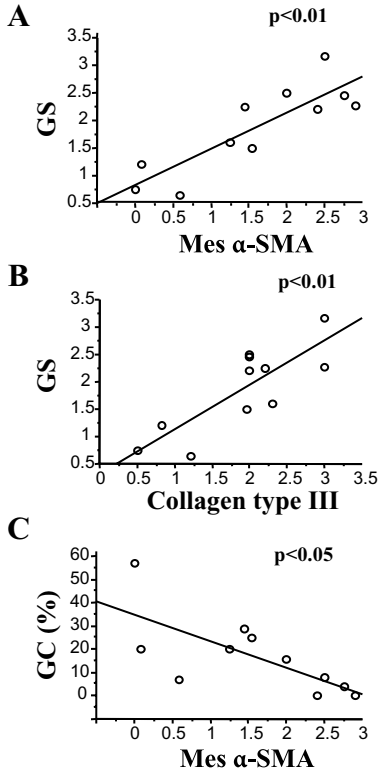

Fig. 5. *Relationship of GS with Mes α-SMA (A) and Col. III (B), and relationship of GC with Mes α-SMA (C). GS was positively correlated with Mes α-SMA and Col. III. GC was inversely correlated with Mes α-SMA.*

by using an appropriately sized cuff and a sphygmomanometer. Patients rested for more than 30 min before blood pressure measurement. Blood pressures were assessed more than 3 times and the values were averaged. SBP from 140 mmHg to 179 mmHg was defined as mild-to-moderate hypertension and SBP over 180 mmHg was defined as severe hypertension according to the Sixth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (*13*). Table 1 shows clinical and pathological data of the patients arranged in order of ascending SBP value. With regard to malignant hypertension, 2 patients had a DBP of more than 130 mmHg (cases 9, 11) and another 1 patient (case 8) had grade III hypertensive retinopathy according to the Keith-Wagener classification. In addition, 3 patients (cases 2, 9, 11) showed heavy proteinuria (PTN>3.5 g/day), and their proteinuria was ameliorated immediately after administration of antihypertensive drugs. Four patients showed necrotizing arterial lesions (cases 1, 6, 7, 11) suggestive of malignant nephrosclerosis.

As a control, biopsies were obtained from a lesion-free portion of the kidneys of 7 patients with renal cell carcinoma and without hypertension and stained for the same antibody. The mean values of the control group were: age, 46 years; SBP/DBP, 115/75 mmHg; sCr, 0.82 mg/dl. Informed consent for the anonymous use of biopsy samples for clinical research was obtained from each subject.

Immunohistochemical Studies

Formalin-fixed paraffin-embedded tissues were available from all 11 cases. The immunohistochemistry technique was performed with the following monoclonal antibodies: α-SMA (DAKO A/S, Glostrup, Denmark), Col. III (LSL Co., Ltd., Tokyo, Japan), Bax (Santa Cruz Biotechnology, Santa Cruz, USA), and Bcl-2 (DAKO A/S).

To reveal the antigens, Col. III sections were pre-treated in a trypsin solution and Bax sections were autoclaved. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, after which the sections were coated with protein blocking agent (PBA: DAKO North America Inc., Carpinteria, USA) to block non-specific reactions. The sections were then coated with the primary antibody at 4°C overnight, rinsed with PBS, and incubated with the secondary antiserum antibody (Envision: DAKO North America Inc.). Finally, the peroxidase reaction was developed with the use of diaminobenzidine. The slides were counterstained with Mayer's hematoxylin.

Histological Assessments

Two pathologists reviewed the slides separately. GO was taken as the percentage of total glomeruli that were solidified. The degree of GS was scored for each glomerulus, because GS was observed for focal and segmental distribution. On the other hand, we scored the GC as the percentage of the total glomeruli that were collapsed, because we recognized GC not segmentally but globally with an all-or-none distribution. Arterio-arteriolosclerosis was evaluated as the presence of hyalinization or thickening of the intimal-medial wall in the small arteries and arterioles. The number of arteries with arterio-arteriolosclerosis per the total number of arteries observed is indicated as the A. sclerosis value in Table 1. The number of arteries and arterioles with onion-skin lesions or fibrinoid necrosis (A. necrosis) is also shown. Using Image J software, interstitial fibrosis (IF) was scored as the percentage of fibrotic area by measuring the percentage of the blue area in each specimen after Masson-trichrome staining. Each glomerulus was scored on a 0 to 4 scale (GS) and a 0 to 3 scale (α-SMA, Col. III, Bax, Bcl-2), based on the degree of staining. Specifically, segmental GS was scored on a 0 to 4 scale (0, no sclerosis; 1+, less than one-third of glomeruli; 2+, one-third to two-thirds of glomeruli; 3+, more than two-thirds of glomeruli; 4+, complete sclerosis). Mesangial α-SMA (Mes α-SMA) was scored on a 0 to 3 scale (0, no lesion present; 1+, one-third of the mesangial area; 2+, one-third to two-thirds of the mesangial area; 3+, more than two-thirds of the mesangial area). Col. III was scored on a 0 to 3 scale (0, no lesion present; 1+, less than 10% of the mesangial area; 2+, less than 50% of the mesangial area; 3+, more than 50% of the mesangial area). Bax staining was evaluated independently on endothelial cells and epithelial podocytes. Podocyte Bax was scored on a 0 to 3 scale (0, no lesion present; 1+, less than 20% of the podocytic area; 2+, 20–50% of the podocytic area; 3+, more than 50% of the podocytic area). Endothelial Bax was scored on a 0 to 3 scale (0, no lesion present; 1+, less than 20% of the endothelial area; 2+, 20–50% of the endothelial area; 3+, more than 50% of the endothelial area). Because Bcl-2 staining was weak, the number of positive-staining nuclei per glomerulus was scored for Bcl-2. Then, the average score per slide was expressed as the sum of the grades of each glomerulus divided by the total number of glomeruli. The relationships between these clinical and morphological parameters in all 11 patients were examined by simple linear regression analysis and unpaired *t*-test. Finally, to adjust for the possible influence of age and proteinuria, multivariate analysis was performed. *p* values less than 0.05 were considered to have statistical significance.

Results

Relationships between Histological Findings and Clinical Data

The mean±SD scores were as follows: GS, 1.9±0.8; GC, 16.9±16.6%; GO, 33.4±15%; and IF, 86.6±14%. GO was positive in all 11 cases and GC was positive in 10 out of 11 cases. Figure 1 shows characteristic staining of GS, GO and GC. The samples were positive for A. necrosis in 4 of 11 patients. On the other hand, the 7 patients of the control group showed no significant glomerular lesions of hypertensive nephrosclerosis, except for 1 patient who had a GO score of 5% (1/20 glomeruli). In a simple linear analysis of all patients, sCr was positively correlated with GC ($p<0.05$) and IF ($p<0.05$), and showed no correlation with GS and GO (Fig. 2A, B). However, after adjusting for age and proteinuria, IF arose as the independent factor which contributed to the increased sCr. In addition, the patients with A. necrosis showed significantly higher sCr (2.3±0.9 mg/dl) than those without A. necrosis (1.2±0.6 mg/dl) ($p<0.05$) by unpaired *t*-test.

As shown in Table 1, GS was frequently observed in the mild-to-moderate hypertension group (140≤SBP<180 mmHg), whereas GC was frequently observed in the severe hypertension group (SBP≥180 mmHg). In relationship to blood pressures, GS showed a negative correlation with SBP ($p<0.05$), but this relation disappeared after adjusting for age and proteinuria (Fig. 2C). On the other hand, GC showed a positive correlation with SBP ($p<0.01$) (Fig. 2D). GO was significantly correlated with GS ($p<0.01$) but was not correlated with GC.

The results described above were confirmed upon applying multivariate analysis employing age and proteinuria as the parameters.

Relationships between Clinicopathological Findings and Synthetic and Secreting Phenotypic Changes

The control group showed no significant staining of α-SMA and Col. III. In contrast, the patient group showed staining of α-SMA and Col. III in the mesangial and interstitial area. The scores (mean±SD) were 1.6±1.0 for Mes α-SMA, and 1.9±0.8 for Col. III. Figure 3 shows a characteristic result of staining for Mes α-SMA and Col. III.

In the clinical comparison, SBP was inversely correlated with Mes α-SMA ($p<0.01$) and Col. III ($p<0.05$) (Fig. 4). In the histological comparison, GS was positively correlated with Mes α-SMA and Col. III ($p<0.01$) (Fig. 5A, B). In contrast, GC was inversely correlated with Mes α-SMA ($p<0.05$) (Fig. 5C). In addition, Col. III was positively correlated with Mes α-SMA ($p<0.01$). As a whole, these phenotypic markers showed similar tendencies in relationships with

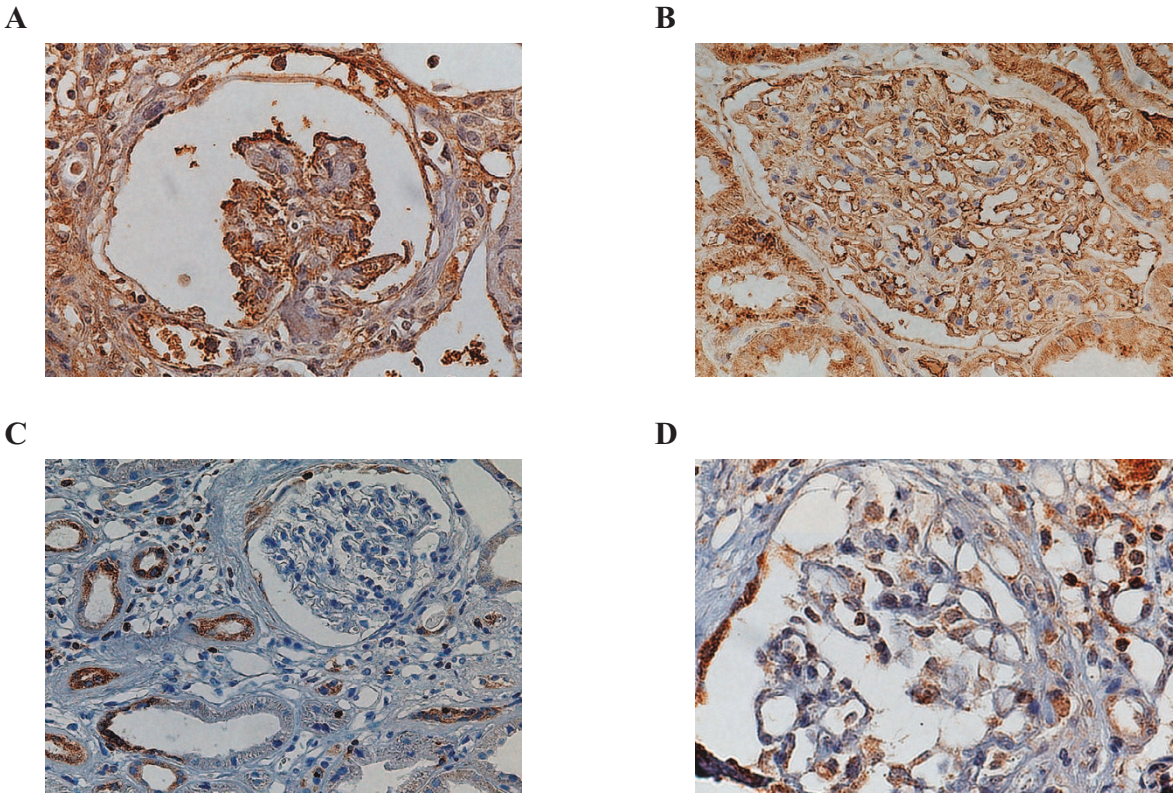

Fig. 6. *Characteristic findings of podocyte Bax (A), endothelial Bax (B), Bcl-2 staining in denatured tubules (C) and Bcl-2 staining in glomerular podocytes and epithelial cells in Bowman's capsule (D).*

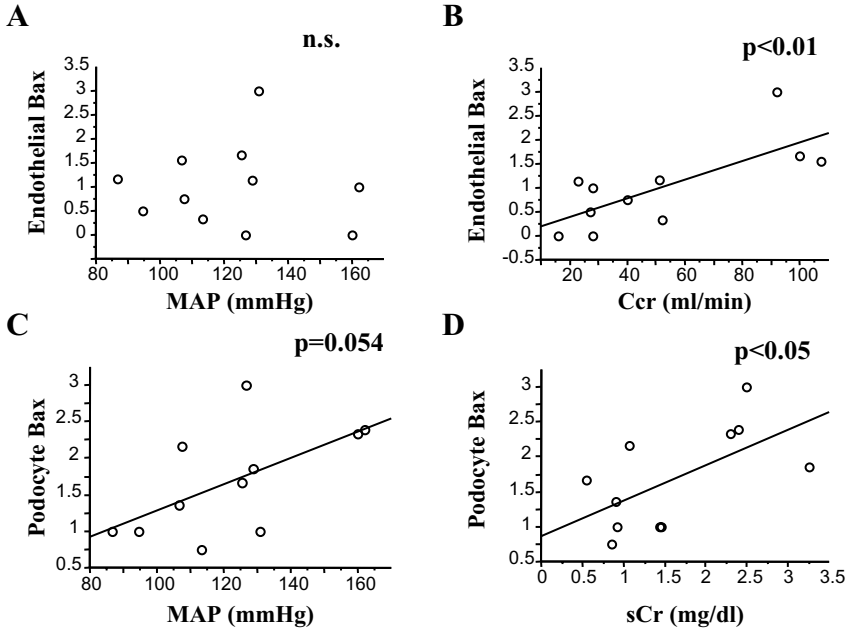

Fig. 7. *Relationships of endothelial Bax with MAP (A), Ccr (B) and relationships of podocyte Bax with MAP (C), sCr (D). Endothelial Bax was positively correlated with Ccr but was not correlated with MAP. Podocyte Bax was positively correlated with MAP and sCr.*

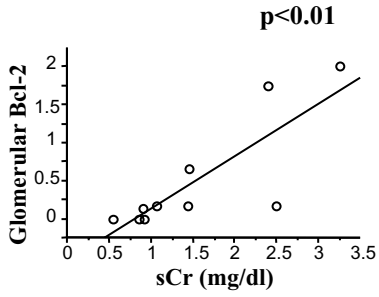

Fig. 8. *Relationships of glomerular Bcl-2 with sCr. Glomerular Bcl-2 was positively correlated with sCr.*

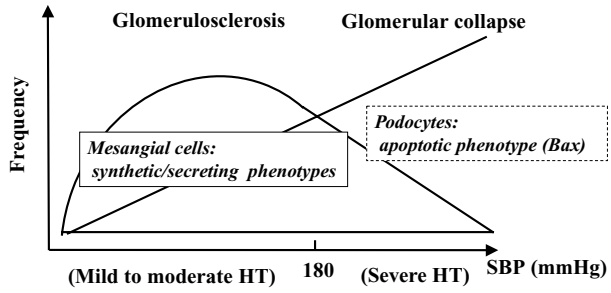

Fig. 9. *Hypothetical schema between SBP and glomerular pathology and phenotypic changes (details are provided in the text).*

GS, GC and SBP.

After adjustment for age and proteinuria employing multivariate analysis, the results described above were confirmed.

Relationships between Clinical Findings and Apoptotic Markers

The control group showed no significant staining of Bax and Bcl-2. In the patient group, Bcl-2 was stained mainly on tubular epithelial cells and interstitial infiltrating mononuclear cells, with weak glomerular staining. Glomerular Bax was positive in podocytes and endothelial cells, to a similar extent as in tubular epithelial cells. However, the mesangial Bax expression was slight. The scores (mean±SD) were 1.0±0.7 for endothelial Bax, 1.5±0.8 for podocyte Bax, and 0.5±0.8 for Bcl-2. Figure 6 shows characteristic results of staining for endothelial Bax, podocyte Bax and Bcl-2.

Endothelial Bax showed a positive correlation with Ccr ($p<0.01$) and no relationship to MAP (Fig. 7A, B). In contrast, podocyte Bax was positively correlated with sCr ($p<0.05$) and tended to correlate with MAP ($p=0.054$) (Fig. 7C, D). In addition, by unpaired *t*-test, the patients with A. necrosis showed significantly stronger podocyte Bax staining (2.3±0.5) compared to those without A. necrosis (1.3±0.6) ($p<0.05$). Glomerular Bcl-2 expression was correlated with sCr ($p<0.01$) (Fig. 8). On multivariate analysis using age and proteinuria as the parameters, the same results were obtained.

Discussion

All of the patients in this study were diagnosed as having hypertensive nephrosclerosis without other apparent renal diseases. Previous studies on benign nephrosclerosis reported that the extent of GS was correlated with the clinical severity, as measured by SBP and other parameters, and with the prognosis (*14*, *15*). The study which was performed by Fogo *et al*. (*14*) was particularly targeted patients with mild-to-moderate renal insufficiency in the absence of nephrotic-range proteinuria with the exclusion of patients having blood pressures >140/90 mmHg, because biopsy was contraindicated in these patients. In contrast to this previous study, a total 8 out of 11 patients in our study showed findings characteristic of malignant nephrosclerosis, including severe or nephrotic-range proteinuria (4 patients) and arterial necrosis (4 patients). Also, 3 patients showed DBP of more than 130 mmHg (2 patients) or severe hypertensive retinopathy (1 patient), which are suggestive of malignant hypertension.

In our study, the severity of GS was not correlated with the extent of hypertension, but as shown in Table 1, GS was frequently seen in subjects with mild-to-moderate hypertension (140≤SBP<180 mmHg). We hypothesize that in mild-to-moderate hypertension, glomerular mesangial cells are stimulated by the proliferation of the mesangial matrices. Analysis of the phenotypic change of glomerular cells revealed that the synthetic and secreting phenotypes Mes α-SMA and Col. III were positively correlated with GS. This suggests that GS is a reflection of these synthetic and secreting phenotypic changes. Furthermore, the synthetic and secreting phenotypes decreased as the severity of systolic hypertension increased. On the other hand, as such synthetic and secreting phenotypes decreased, GC tended to increase.

It is noteworthy that there was a positive correlation between SBP and GC. Considering the pathogenic mechanisms of GC, glomerular hypoperfusion due to arteriolar narrowing has been considered as a major cause of GC formation (*1*). In addition, hypertensive shear stress may cause endothelial cell damage leading to mesangiolysis with capillary ballooning, which may cause podocyte damage in association with high intracapillary pressure (*16*, *17*). As a post-glomerular factor, tubular degeneration often causes the formation of atubular glomeruli, which in turn contributes to increased extracapillary glomerular pressure. Collectively, these factors may collaborate in the development of GC. Furthermore, the correlation between sCr and GC in the current study may support the conclusion that collapsed glomeruli cannot retain their filtration function.

In the present study, Bax expression appeared to be stronger than Bcl-2 expression, which indirectly lends support to other observations of acceleration of apoptosis in hyperten-

sive nephrosclerosis (*11*, *12*). Furthermore, podocyte Bax expression was significantly correlated with sCr and tended to correlate with MAP, although endothelial Bax expression was stronger in patients with preserved Ccr. We speculate that the magnitude of blood pressures has an effect on the development of apoptosis between endothelial cells and podocytes. Namely, in the mild-to-moderate hypertensive state, apoptosis may occur in endothelial cells. On the other hand, in the severe hypertensive state, the progression of mesangiolysis which causes mesangial expansion and capillary ballooning may trigger apoptosis in podocyte (*16*).

It has been reported that altered podocyte structure is associated with hypertension and low glomerular filtration rate (*17*). The present study contributes the new insight that apoptotic change in podocytes may be one of the major causes of the pathogenesis of progressive hypertensive nephrosclerosis.

Essentially, we consider GS and GC to be two separate processes, rather than successive steps in GO, although, in the present study, there were two cases having an admixture of two basic types of GS and GC. In conclusion, the current study suggests that in mild-to-moderate hypertension, GS occurs predominantly with mesangial synthetic and secreting phenotypic changes; whereas, in severe hypertension, GC increases in association with podocyte apoptotic phenotypic changes (Fig. 9).

References

1. Helmcben U, Wenzel UO: Benign and malignant nephrosclerosis and renovascular disease, in Tisher CC, Brenner BM: Renal Pathology. Philadelphia, Lippincott, 1994, pp 1201–1236.
2. Olson JL: Hypertension: essential and secondary forms, in Jennette JC, Olson JL, Schwartz MM, Silva FG: Heptinstall's Pathology of the Kidney, 5th ed. Philadelphia, Lippincott, 1998, pp 943–1001.
3. Nakaya H, Sasamura H, Kitamura Y, *et al*: Effects of angiotensin inhibitors on renal injury and angiotensin receptor expression in early hypertensive nephrosclerosis. *Hypertens Res* 1999; **22**: 303–312.
4. Luft FC: Hypertensive nephrosclerosis: update. *Curr Opin Nephrol Hypertens* 2004; **13**: 147–154.
5. Inada H, Ono H, Minami J, Ishimitsu T, Matsuoka H: Nipradilol prevents L-NAME–exacerbated nephrosclerosis with decreasing of caspase-3 expression in SHR. *Hypertens Res* 2002; **25**: 433–440.
6. Rupprecht HD, Schocklmann HO, Sterzel RB: Glomerular mesangial cells, in Neilson EG, Couser WG: Immunologic Renal Disease, 2nd ed. Philadelphia, Lippincott, 2001, pp 657–691.
7. Sanders PW, Wang PX: Activation of the Fas/Fas ligand pathway in hypertensive renal disease in Dahl/Rapp rats. *BMC Nephrol* 2002; **3**: 1.
8. Sugiyama H, Kashihara N, Makino H, Yamasaki Y, Ota Z: Apoptosis in glomerular sclerosis. *Kidney Int* 1996; **49**: 103–111.
9. Ono H, Ono Y, Takanohashi A, Matsuoka H, Frohlich ED: Apoptosis and glomerular injury after prolonged nitric oxide synthase inhibition in spontaneously hypertensive rats. *Hypertension* 2001; **38**: 1300–1306.
10. Ono H, Saitoh M, Ono Y, Ishimitu T, Matsuoka H: Imidapril improves L-NAME–exacerbated nephrosclerosis with TGF-beta 1 inhibition in spontaneously hypertensive rats. *J Hypertens* 2004; **22**: 1389–1395.
11. Ying WZ, Wang PX, Sanders PW: Induction of apoptosis during development of hypertensive nephrosclerosis. *Kidney Int* 2000; **58**: 2007–2017.
12. Ying WZ, Sanders PW: Cytochrome c mediates apoptosis in hypertensive nephrosclerosis in Dahl/Rapp rats. *Kidney Int* 2001; **59**: 662–672.
13. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Arch Intern Med* 1997; **157**: 2413–2446.
14. Fogo A, Breyer JA, Smith MC, *et al*, AASK Pilot Study Investigators: Accuracy of the diagnosis of hypertensive nephrosclerosis in African Americans: a report from the African American Study of Kidney Disease (AASK) Trial. *Kidney Int* 1997; **51**: 244–252.
15. Takebayashi S, Kiyoshi Y, Hisano S, *et al*: Benign nephrosclerosis: incidence, morphology and prognosis. *Clin Nephrol* 2001; **55**: 349–356.
16. Kritz W, Kobayashi N, Elger M: New aspects of podocyte structure, function, and pathology. *Clin Exp Nephrol* 1998; **2**: 85–99.
17. Kretzler M, Koeppen-Hagemann I, Kriz W: Podocyte damage is a critical step in the development of glomerulosclerosis in the uninephrectomised-desoxycorticosterone hypertensive rat. *Virchows Arch* 1994; **425**: 181–193.