



Association of renal arteriosclerosis and hypertension with renal and cardiovascular outcomes in Japanese type 2 diabetes patients with diabetic nephropathy

Miho Shimizu^{1,2,3,*} , Kengo Furuichi^{1,2}, Tadashi Toyama^{1,2}, Tomoaki Funamoto^{1,2}, Shinji Kitajima^{1,2}, Akinori Hara¹, Yasunori Iwata^{1,2}, Norihiko Sakai^{1,2}, Toshinari Takamura⁴, Kiyoki Kitagawa⁵, Mitsuhiro Yoshimura⁶, Shuichi Kaneko², Hitoshi Yokoyama⁷, Takashi Wada^{1,8} , Kanazawa Study Group for Renal Diseases and Hypertension

¹Division of Nephrology, Kanazawa University Hospital, ²Department of System Biology, Graduate School of Medical Sciences, ³Health Service Center, ⁴Department of Endocrinology and Metabolism, Graduate School of Medical Sciences, Kanazawa University, ⁵Division of Internal Medicine, National Hospital Organization Kanazawa Medical Center, Kanazawa, ⁶Division of Internal Medicine, Noto General Hospital, Nanao, ⁷Division of Nephrology, Kanazawa Medical University, Uchinada, and ⁸Department of Nephrology and Laboratory Medicine, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan

Keywords

Diabetic nephropathy, Hypertension, Renal arteriosclerosis

*Correspondence

Miho Shimizu
Tel: +81-76-264-5251
Fax: +81-76-234-4044
E-mail address:
mshimizu@staff.kanazawa-u.ac.jp

J Diabetes Investig 2019; 10: 1041–1049

doi: 10.1111/jdi.12981

ABSTRACT

Aims/Introduction: The present retrospective study investigated the impact of renal arteriosclerosis (AS) and hypertension (HT) on long-term renal and cardiovascular outcomes in Japanese type 2 diabetes patients with biopsy-proven diabetic nephropathy.

Materials and Methods: A total of 185 patients were enrolled. Patients were divided into four groups stratified by renal AS status and the presence of HT. The outcomes for this study were the first occurrence of renal events (a need for dialysis or a 30% decline in estimated glomerular filtration rate from baseline) and cardiovascular events (cardiovascular death, non-fatal myocardial infarction, coronary intervention or non-fatal stroke).

Results: The proportion of renal AS scores ≥ 1 was 88.3% among patients with normal-range blood pressure (BP) and 95.4% among patients with HT. During a mean follow-up period of 7.6 years, 129 episodes of renal composite events and 55 episodes of cardiovascular events were observed. Compared with patients with no renal AS and normal-range BP, a renal AS score ≥ 1 increased the risk of renal composite events with a multivariable-adjusted hazard ratio of 3.21 (95% confidence interval [95% CI] 1.27–8.14) in patients with normal-range BP and 4.99 (95% CI 1.98–12.54) in patients with HT, whereas renal AS score ≥ 1 increased the risk of cardiovascular events with a multivariable-adjusted hazard ratio of 6.06 (95% CI 1.24–29.61) in patients with normal-range BP and 10.02 (95% CI 1.92–52.39) in patients with HT.

Conclusions: Renal AS was associated with increasing risks for renal composite events and cardiovascular events in both normal-range BP and HT. The risks of renal composite events and cardiovascular events were the highest in both renal AS and HT.

INTRODUCTION

Hypertension (HT) is a major risk factor for both microvascular and macrovascular complications related to diabetes¹. Furthermore, numerous studies have shown that antihypertensive therapy reduces the risk of these complications¹.

Renal arteriosclerosis (AS) is not a specific pathological finding in diabetic nephropathy². However, we have previously reported that renal AS score evaluated according to the pathological classification of diabetic nephropathy by the Research Committee of the Renal Pathology Society² is associated with renal and cardiovascular outcomes in Japanese type 2 diabetes patients with diabetic nephropathy^{3,4}. Although previous studies

Received 24 July 2018; revised 13 November 2018; accepted 2 December 2018

have clarified the close relationships between renal AS and HT^{5–8}, the relative contributions of renal AS and HT to renal and cardiovascular outcomes in type 2 diabetes patients with diabetic nephropathy is not well understood.

Therefore, we carried out a long-term retrospective study to evaluate the interaction of renal AS with HT on the risks for renal and cardiovascular outcomes in Japanese type 2 diabetes patients with biopsy-proven diabetic nephropathy.

METHODS

Study Population

A total of 185 type 2 diabetes patients with biopsy-proven diabetic nephropathy who were diagnosed at Kanazawa University Hospital or Kanazawa Medical Center in Kanazawa, Japan, between 1985 and 2017 were included in the present study. The diagnosis of diabetes was determined by medical history and/or the criteria of the Japanese Diabetic Society as follows: (i) fasting plasma glucose level of ≥ 126 mg/dL (≥ 7.0 mmol/L); (ii) 2-h value of ≥ 200 mg/dL (≥ 11.1 mmol/L) in 75-g oral glucose tolerance test; (iii) casual plasma glucose level of ≥ 200 mg/dL (≥ 11.1 mmol/L); or (iv) hemoglobin A1c $\geq 6.5\%$ ⁹. A renal biopsy was carried out for the precise diagnosis of kidney lesions with the consent of each patient. The diagnosis of diabetic nephropathy was confirmed by typical pathological features consistent with diabetic nephropathy using renal biopsy, including light microscopy, electron microscopy and immunofluorescence examination. Patients with other glomerular diseases concomitant with diabetic nephropathy were excluded from this study. The study protocol was approved by the ethics committees of Kanazawa University and Kanazawa Medical Center.

Clinical Examinations

Baseline was defined as the time of renal biopsy. Age, sex, serum creatinine, estimated glomerular filtration rate (eGFR), 24-h urinary protein excretion, systolic blood pressure (BP), diastolic BP, duration of diabetes, presence of diabetic retinopathy, hemoglobin A1c, total cholesterol and body mass index were obtained as clinical variables at baseline. eGFR was calculated using the equation by the Japanese Society of Nephrology¹⁰. HT was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg irrespective of antihypertensive medication¹¹.

Pathological Examinations

For light microscopic examination, renal biopsy specimens were fixed in 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin and sliced into sections 4- μ m thick. These specimens were stained with periodic acid–Schiff (PAS) reagent, periodic acid silver methenamine, hematoxylin–eosin and Mallory–Azan, and examined by light microscopy. The severity of renal AS was scored from 0 to 2 according to the description by Tervaert *et al.*² as follows: score 0, no intimal thickening (Figure 1a,d); score 1, intimal thickening less than the thickness

of the media (Figure 1b,e); and score 2, intimal thickening greater than the thickness of the media (Figure 1c,f)³. The severity of the diffuse lesions in the glomeruli was scored from 0 to 4 according to the description by Gellman *et al.* (score 0, all glomeruli appear normal; score 1, local lesions present within each glomerulus and focal lesions present within the kidney; score 2, diffuse mesangial thickening within the glomerulus and generalized throughout the kidney; and score 3, narrowed capillary lumina and local obliteration)^{3,12}. Nodular lesion, exudative lesion and mesangiolysis were defined as the presence or absence in each specimen^{3,13}. The severity of interstitial fibrosis and tubular atrophy (IFTA) was scored from 0 to 3 according to the description by Tervaert *et al.* (score 0, no IFTA; score 1, $<25\%$; score 2, $25\text{--}50\%$; score 3, $>50\%$)^{2,3}. The severity of interstitial cell infiltration was scored from 0 to 2 according to the description by Tervaert *et al.* (score 0, absent; score 1, infiltration only in relation to IFTA; score 2, infiltration in areas without IFTA)^{2,3}. The severity of arteriolar hyalinosis was scored from 0 to 3 according to the description by Takazakura *et al.* (score 0, a normal appearance without PAS-positive deposits; score 1, a light PAS-positive thickening observed, but involving less than half of the circumference of the arteriole in many arterioles; score 2, numerous moderately thickened vessel walls with PAS-positive deposition without apparent luminal narrowing; score 3, a heavy thickening of the majority of the vessel walls with luminal narrowing or obliteration)^{3,14}. Renal tissue specimens were assessed by four nephrologists.

Outcomes

The outcomes for the present study were the first occurrence of renal events (a need for dialysis or a 30% decline in eGFR from baseline) and cardiovascular events (cardiovascular death, non-fatal myocardial infarction, coronary intervention or non-fatal stroke). A $\geq 30\%$ decline in eGFR was selected as the renal outcome of this study based on a series of meta-analyses of clinical trials and observational studies that revealed a relationship between lesser declines than a halving in eGFR and end-stage renal disease (ESRD)¹⁵. We also reported that a $\geq 30\%$ decline in eGFR over 1 or 2 years adds prognostic information about the risk for ESRD requiring dialysis in type 2 diabetes patients with macroalbuminuria¹⁶. None of the patients received kidney transplantation during follow up. The patients were followed until the end of 2017 or death.

Statistical Analysis

Data are expressed as the mean \pm standard deviation for continuous variables and the number (percentage) for categorical variables. Mann–Whitney *U*-tests, Kruskal–Wallis tests and χ^2 -tests were applied to identify differences in continuous and categorical variables. The cumulative incidences of the outcomes were estimated using the Kaplan–Meier method and were compared by log-rank test. The hazard ratio (HR) and their 95% confidence intervals (CIs) of different groups

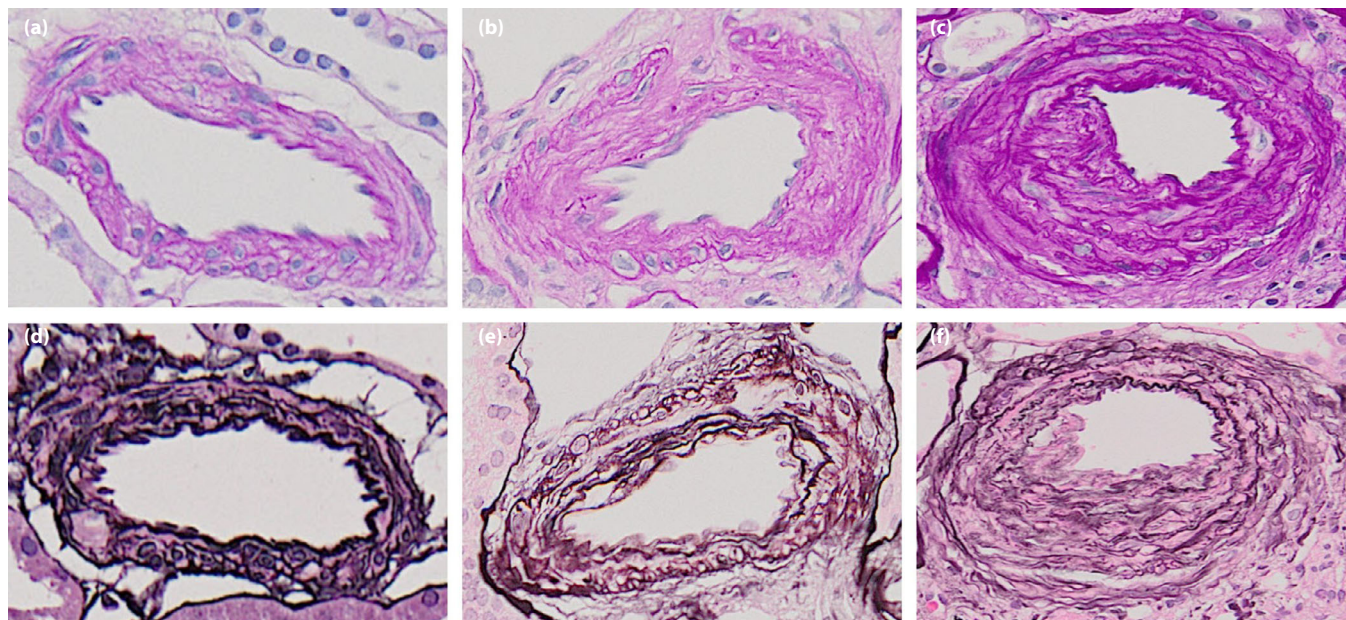


Figure 1 | Representative microscopic findings of renal arteriosclerosis in diabetic nephropathy. Various grades of renal arteriosclerosis (score 0–2). (a–c) Periodic acid–Schiff stain (magnification: $\times 200$). (d–f) Periodic acid silver methenamine stain (magnification: $\times 200$). Renal arteriosclerosis was semiquantitatively assessed as follows: (a,d) score 0, absence of intimal thickening; (b,e) score 1, intimal thickening less than the media thickness; and (c,f) score 2, intimal thickening greater than the media thickness.

stratified by renal AS status and the presence of HT on each outcome were calculated using univariable and multivariable Cox proportional hazards model analyses. Patients with no renal AS (score 0) and normal-range BP were served as the reference group. Baseline clinical and pathological variables were incorporated as covariates in the stepwise procedure. SPSS version 24 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A two-sided $P < 0.05$ was considered to show statistical significance.

RESULTS

Baseline clinical characteristics according to renal AS

A total of 185 patients were enrolled in the present study, including 60 women and 125 men with a mean age of 59.0 years. At baseline, 14, 83 and 88 patients were classified into renal AS score 0, 1 and 2, respectively. Baseline clinical data according to renal AS score are listed in Table 1. Compared with no renal AS (score 0), renal AS score ≥ 1 was associated with higher age, higher prevalence of male, higher serum creatinine, lower eGFR and higher prevalence of diabetic retinopathy. Urinary protein excretion, systolic BP, diastolic BP, prevalence of HT, duration of diabetes, hemoglobin A1c, total cholesterol and body mass index did not differ significantly between scores of renal AS.

Clinical and Pathological Characteristics According to Renal AS Status and the Presence of HT

Next, we divided the patients into four groups according to renal AS score and the presence of HT as follows: no renal AS

and normal-range BP (systolic BP < 140 mmHg and diastolic BP < 90 mmHg; $n = 9$); no renal AS and HT ($n = 5$); renal AS score ≥ 1 and normal-range BP ($n = 68$); and renal AS score ≥ 1 and HT ($n = 103$). The proportion of patients with renal AS score ≥ 1 was 88.3% (68 of 77) among those with normal-range BP, and 95.4% (103 of 108) among those with HT. Clinical and pathological characteristics according to renal AS status and the presence of HT are listed in Table 2. Patients with renal AS score ≥ 1 and HT were associated with higher age, higher serum creatinine, lower eGFR, higher urinary protein excretion, higher systolic BP, higher diastolic BP, higher prevalence of diabetic retinopathy, higher total cholesterol, higher diffuse lesion score, higher prevalence of nodular lesion, higher IFTA score, higher interstitial cell infiltration score, higher arteriolar hyalinosis score and higher AS score. Sex, duration of diabetes, hemoglobin A1c, body mass index, prevalence of exudative lesion and prevalence of mesangiolysis did not differ significantly among groups stratified by renal AS status and the presence of HT.

Prognosis of Renal and Cardiovascular Outcomes According to Renal AS Status and the Presence of HT

The mean follow-up duration was 7.6 years (median 6.3 years, maximum 29.4 years) during 1985–2017. There were a total of 129 renal composite events and 55 cardiovascular events.

The cumulative incidence of renal composite events was different among groups stratified by renal AS status and the presence of HT ($P < 0.01$; Figure 2a). The cumulative incidence of

Table 1 | Baseline clinical characteristics according to renal arteriosclerosis score

	All	Renal AS			P
		Score 0 (n = 14)	Score 1 (n = 83)	Score 2 (n = 88)	
Age (years)	59.0 ± 10.7	46.3 ± 13.5	59.0 ± 10.2	61.0 ± 9.3	<0.01
Male, n (%)	125 (67.6)	8 (57.1)	64 (77.1)	53 (60.2)	<0.05
Serum creatinine (mg/dL)	1.5 ± 1.4	0.8 ± 0.3	1.4 ± 1.3	1.7 ± 1.5	<0.01
eGFR (mL/min/1.73 m ²)	53.8 ± 28.9	86.7 ± 25.9	54.1 ± 26.8	48.2 ± 28.0	<0.01
Urinary protein excretion (g/day)	2.7 ± 2.7	1.7 ± 2.3	3.0 ± 2.8	2.5 ± 2.6	0.05
Systolic BP (mmHg)	143.8 ± 21.2	137.0 ± 11.7	143.9 ± 20.4	144.8 ± 22.1	0.29
Diastolic BP (mmHg)	77.5 ± 12.4	80.1 ± 10.2	76.5 ± 12.6	78.1 ± 12.4	0.55
HT (BP ≥140/90 mmHg), n (%)	108 (58.4)	5 (35.7)	53 (63.9)	50 (56.8)	0.13
Diabetes duration (years)	12.3 ± 8.1	9.3 ± 7.1	13.6 ± 8.2	11.5 ± 8.1	0.07
Presence of diabetic retinopathy, n (%)	152 (84.9)	7 (50.0)	70 (86.4)	75 (89.3)	<0.01
Hemoglobin A1c (%)	7.6 ± 2.2	7.6 ± 2.3	7.5 ± 2.0	7.7 ± 2.4	0.96
Total cholesterol (mg/dL)	223.0 ± 90.7	208.2 ± 49.2	210.8 ± 61.9	237.0 ± 115.3	0.75
Body mass index (kg/m ²)	23.3 ± 3.6	25.3 ± 4.5	22.8 ± 3.5	23.3 ± 3.5	0.08

AS, arteriosclerosis; BP, blood pressure; eGFR, estimated glomerular filtration rate; HT, hypertension.

Table 2 | Baseline clinical and pathological characteristics in groups stratified by renal arteriosclerosis status and the presence of hypertension

	AS(-)/HT(-) (n = 9)	AS(-)/HT(+) (n = 5)	AS(+)/HT(-) (n = 68)	AS(+)/HT(+) (n = 103)	P
Clinical variables					
Age (years)	45.2 ± 16.4	48.2 ± 7.1	58.9 ± 10.9	60.8 ± 8.9	<0.01
Male, n (%)	4 (44.4)	4 (80.0)	52 (76.5)	65 (63.1)	0.11
Serum creatinine (mg/dL)	0.7 ± 0.3	0.8 ± 0.3	1.4 ± 1.1	1.7 ± 1.6	<0.01
eGFR (mL/min/1.73 m ²)	88.8 ± 24.7	83.0 ± 30.4	59.2 ± 31.5	45.7 ± 23.2	<0.01
Urinary protein excretion (g/day)	1.3 ± 1.8	2.5 ± 3.0	1.8 ± 2.0	3.3 ± 3.0	<0.01
Systolic BP (mmHg)	124.7 ± 8.1	159.2 ± 15.8	125.0 ± 10.9	157.2 ± 16.0	<0.01
Diastolic BP (mmHg)	75.1 ± 9.4	89.2 ± 13.2	70.6 ± 9.7	81.8 ± 12.1	<0.01
Diabetes duration (years)	9.7 ± 6.7	8.6 ± 7.8	11.6 ± 7.7	13.1 ± 8.5	0.33
Presence of diabetic retinopathy, n (%)	5 (55.6)	2 (40.0)	54 (84.4)	91 (90.1)	<0.01
Hemoglobin A1c (%)	7.8 ± 2.8	7.1 ± 2.2	7.8 ± 2.4	7.5 ± 2.0	0.95
Total cholesterol (mg/dL)	199.9 ± 34.2	223.2 ± 18.6	196.8 ± 67.3	243.5 ± 104.9	<0.01
Body mass index (kg/m ²)	24.8 ± 3.1	26.0 ± 6.7	23.1 ± 3.5	23.1 ± 3.4	0.26
Pathological variables					
Diffuse lesion (0–4)	1.2 ± 0.8	1.8 ± 1.6	2.2 ± 0.9	2.5 ± 0.8	<0.01
Presence of nodular lesion, n (%)	1 (11.1)	2 (40.0)	34 (50.7)	62 (61.4)	<0.05
Presence of exudative lesion, n (%)	3 (33.3)	1 (20.0)	26 (38.8)	42 (41.6)	0.77
Presence of mesangiolysis, n (%)	1 (11.1)	2 (40.0)	14 (20.9)	39 (38.6)	0.05
IFTA (0–3)	1.0 ± 1.0	1.4 ± 1.1	1.8 ± 0.9	2.2 ± 0.8	<0.01
Interstitial cell infiltration (0–2)	0.6 ± 0.7	0.8 ± 0.4	1.1 ± 0.5	1.2 ± 0.4	<0.01
Arteriolar hyalinosis (0–3)	0.8 ± 1.1	1.2 ± 1.1	2.1 ± 1.0	2.2 ± 0.8	<0.01
AS (0–2)	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 0.5	1.5 ± 0.5	<0.01

AS, arteriosclerosis; BP, blood pressure; eGFR, estimated glomerular filtration rate; HT, hypertension; IFTA, interstitial fibrosis and tubular atrophy.

renal composite events in patients with renal AS score ≥1 and HT was higher than in those with renal AS score ≥1 and normal-range BP ($P < 0.01$). However, the cumulative incidence of renal composite events showed no significant differences

between normal-range BP and HT in patients with no renal AS.

The cumulative incidence of cardiovascular events was also different among groups stratified by renal AS status and the

presence of HT ($P < 0.01$; Figure 2b). The cumulative incidence of cardiovascular events in patients with renal AS score ≥ 1 and HT was higher than in those with renal AS score ≥ 1 and normal-range BP ($P < 0.05$). However, the cumulative incidence of cardiovascular events showed no significant differences between normal-range BP and HT in patients with no renal AS.

Risks of Renal and Cardiovascular Outcomes According to Renal AS Status and the Presence of HT

Table 3a shows the estimated HRs of renal composite events relative to the group of no renal AS and normal-range BP for

other groups stratified by renal AS status and the presence of HT based on univariable and multivariable Cox regression analyses. Compared with patients with no renal AS and normal-range BP, the risk of renal composite events in patients with renal AS score ≥ 1 and HT was increased (crude HR 7.03, 95% CI 2.41–20.53; $P < 0.01$; adjusted HR 4.99, 95% CI 1.98–12.54; $P < 0.01$). Although not as much as in patients with renal AS score ≥ 1 and HT, the risk of renal composite events in patients with renal AS score ≥ 1 and normal-range BP was also increased (crude HR 3.75, 95% CI 1.30–10.84; $P < 0.05$; adjusted HR 3.21, 95% CI 1.27–8.14; $P < 0.05$). Table 3b shows the estimated HRs of cardiovascular events relative to the group of no renal AS and normal-range BP for other groups stratified by renal AS status and the presence of HT based on univariable and multivariable Cox regression analyses. Compared with patients with no renal AS and normal-range BP, the risk of cardiovascular events in patients with renal AS score ≥ 1 and HT was increased (crude HR 12.26, 95% CI 1.46–103.03; $P < 0.05$; adjusted HR 10.02, 95% CI 1.92–52.39; $P < 0.01$). Although not as much as in patients with renal AS score ≥ 1 and HT, renal AS score ≥ 1 increased the risk of cardiovascular events with a multivariable-adjusted HR of 6.06 (95% CI 1.24–29.61; $P < 0.05$) in patients with normal-range BP.

To confirm these findings, we evaluated the effect of the use of antihypertensive agents or renin–angiotensin system (RAS) inhibitors (angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers) at baseline on the risks of renal and cardiovascular outcomes. The proportion of patients with the use of antihypertensive agents was 44.4% (4/9) among those with no renal AS and normal-range BP, 60.0% (3/5) among those with no renal AS and HT, 62.1% (41/66) among those with renal AS score ≥ 1 and normal-range BP, and 82.5% (85/103) among those with renal AS score ≥ 1 and HT. Compared with patients with no renal AS and normal-range BP, renal AS score ≥ 1 increased the risk of renal composite events with adjusted HRs of 3.19 (95% CI 1.24–8.23; $P < 0.05$) in patients with normal-range BP and 4.76 (95% CI 1.87–12.10; $P < 0.01$) in patients with HT, whereas renal AS score ≥ 1 increased the risk of cardiovascular events with adjusted HRs of 5.57 (95% CI 1.14–27.32; $P < 0.05$) in patients with normal-range BP and 7.71 (95% CI 1.48–40.23; $P < 0.05$) in patients with HT. In a similar way, the proportion of patients taking RAS inhibitors was 33.3% (3/9) among those with no renal AS and normal-range BP, 40.0% (2/5) among those with no renal AS and HT, 43.9% (29/66) among those with renal AS score ≥ 1 and normal-range BP, and 59.2% (61/103) among those with renal AS score ≥ 1 and HT. Compared with patients with no renal AS and normal-range BP, renal AS score ≥ 1 increased the risk of renal composite events with adjusted HRs of 3.06 (95% CI 1.20–7.79; $P < 0.05$) in patients with normal-range BP and 4.84 (95% CI 1.92–12.17; $P < 0.01$) in patients with HT, whereas renal AS score ≥ 1 increased the risk of cardiovascular events with adjusted HRs of 5.66 (95% CI 1.16–

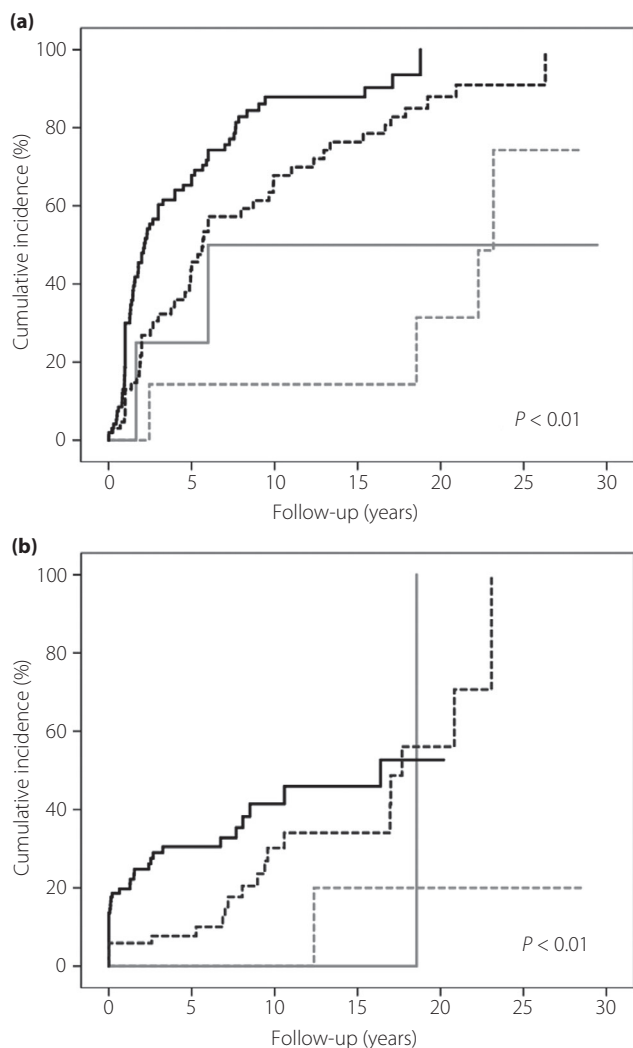


Figure 2 | Cumulative incidence of (a) renal composite events and (b) cardiovascular events compared among groups stratified by renal arteriosclerosis (AS) status and the presence of hypertension. Gray dotted line, no renal AS and normal-range blood pressure ($n = 9$); gray solid line, no renal AS and hypertension ($n = 5$); black dotted line, renal AS score ≥ 1 and normal-range blood pressure ($n = 68$); and black solid line, renal AS score ≥ 1 and hypertension ($n = 103$).

Table 3 | Hazard ratios of renal composite events and cardiovascular events according to renal arteriosclerosis status and the presence of hypertension

Variables	Univariable analysis		Multivariable analysis	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Renal composite events				
AS(-)/HT(+) (vs AS(-)/HT(-))	1.36 (0.25–7.48)	0.73	NS	
AS(+)/HT(-) (vs AS(-)/HT(-))	3.75 (1.30–10.84)	<0.05	3.21 (1.27–8.14)	<0.05
AS(+)/HT(+) (vs AS(-)/HT(-))	7.03 (2.41–20.53)	<0.01	4.99 (1.98–12.54)	<0.01
Age (per 1 year)	1.03 (1.01–1.05)	<0.01	NS	
Male sex	1.18 (0.81–1.73)	0.39	NS	
eGFR (per –1 mL/min/1.73 m ²)	1.02 (1.01–1.03)	<0.01	NS	
Urinary protein excretion (per 1 g/day)	1.32 (1.24–1.40)	<0.01	1.24 (1.15–1.34)	<0.01
Diabetes duration (per 1 year)	1.02 (1.001–1.05)	<0.05	NS	
Presence of diabetic retinopathy	2.51 (1.34–4.70)	<0.01	NS	
Hemoglobin A1c (%)	0.94 (0.87–1.02)	0.16	NS	
Total cholesterol (per 1 mg/dL)	1.004 (1.002–1.006)	<0.01	NS	
Body mass index (per 1 kg/m ²)	0.97 (0.92–1.02)	0.20	NS	
Diffuse lesion (per 1 score)	1.75 (1.46–2.11)	<0.01	1.33 (1.05–1.68)	<0.05
Presence of nodular lesion	3.24 (2.20–4.78)	<0.01	NS	
Presence of exudative lesion	3.12 (2.15–4.52)	<0.01	1.88 (1.19–2.95)	<0.01
Presence of mesangiolytic	2.30 (1.55–3.41)	<0.01	1.75 (1.12–2.73)	<0.05
IFTA (per 1 score)	1.57 (1.28–1.92)	<0.01	NS	
Interstitial cell infiltration (per 1 score)	1.28 (0.93–1.77)	0.13	NS	
Arteriolar hyalinosis (per 1 score)	1.28 (1.06–1.54)	<0.05	NS	
Cardiovascular events				
AS(-)/HT(+) (vs AS(-)/HT(-))	4.42 (0.25–77.79)	0.31	NS	
AS(+)/HT(-) (vs AS(-)/HT(-))	6.57 (0.80–54.13)	0.08	6.06 (1.24–29.61)	<0.05
AS(+)/HT(+) (vs AS(-)/HT(-))	12.26 (1.46–103.03)	<0.05	10.02 (1.92–52.39)	<0.01
Age (per 1 year)	1.04 (1.01–1.07)	<0.01	NS	
Male sex	0.87 (0.50–1.52)	0.63	NS	
eGFR (per –1 mL/min/1.73 m ²)	1.01 (1.003–1.02)	<0.05	NS	
Urinary protein excretion (per 1 g/day)	1.06 (0.96–1.17)	0.23	NS	
Diabetes duration (per 1 year)	1.03 (1.00–1.07)	0.06	NS	
Presence of diabetic retinopathy	1.89 (0.78–4.55)	0.16	NS	
Hemoglobin A1c (%)	1.02 (0.91–1.14)	0.73	NS	
Total cholesterol (per 1 mg/dL)	1.00 (0.997–1.003)	0.95	NS	
Body mass index (per 1 kg/m ²)	0.97 (0.89–1.05)	0.46	NS	
Diffuse lesion (per 1 score)	1.05 (0.79–1.40)	0.75	NS	
Presence of nodular lesion	1.06 (0.60–1.87)	0.85	NS	
Presence of exudative lesion	0.76 (0.41–1.41)	0.38	NS	
Presence of mesangiolytic	1.26 (0.68–2.32)	0.47	NS	
IFTA (per 1 score)	1.08 (0.80–1.45)	0.62	NS	
Interstitial cell infiltration (per 1 score)	0.93 (0.56–1.53)	0.77	NS	
Arteriolar hyalinosis (per 1 score)	1.05 (0.80–1.38)	0.74	NS	

AS, arteriosclerosis; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; HT, hypertension; IFTA, interstitial fibrosis and tubular atrophy; NS, not significant.

27.67; *P* < 0.05) in patients with normal-range BP and 9.20 (95% CI 1.77–47.89; *P* < 0.01) in patients with HT. In these analyses, the similar impacts of renal AS score ≥ 1 on renal composite events and cardiovascular events were observed in both normal-range BP and HT, even after adjustment for the use of antihypertensive agents or RAS inhibitors in addition to other baseline clinical and pathological covariates.

DISCUSSION

To the best of our knowledge, this is the first study to determine the impact of renal AS and HT on long-term renal and cardiovascular outcomes in type 2 diabetes patients with diabetic nephropathy. The impact of renal AS score ≥ 1 on renal composite events and cardiovascular events was observed in both normal-range BP and HT. The risks of renal composite events and cardiovascular events were the highest in both renal

AS and HT. These associations remained statistically significant even after adjustment for the use of antihypertensive agents or RAS inhibitors in addition to baseline clinical and pathological covariates.

First, we showed that renal AS score ≥ 1 was associated with higher risks of renal composite events in both normal-range BP and HT. Renal AS has been reported to contribute to the progression of glomerular lesions in diabetic nephropathy^{17,18}. In addition, the present study showed that renal AS coexisting with HT was associated with advanced diffuse lesion, nodular lesion, IFTA, interstitial cell infiltration and arteriolar hyalinosis. We previously reported the structural-functional relationships in type 2 diabetes patients with diabetic nephropathy³. In that study, pathological findings associated with albuminuria (proteinuria), regardless of eGFR, were glomerular lesions (diffuse lesion, nodular lesion, exudative lesion, mesangiolysis), whereas pathological findings associated with low eGFR, regardless of albuminuria (proteinuria), were glomerular lesions (diffuse lesion, nodular lesion), tubulointerstitial lesions (IFTA, interstitial cell infiltration) and vascular lesions (arteriolar hyalinosis, AS). These findings suggest that the impact of a combination of renal AS and HT on diabetic kidney lesions might be associated with poor renal outcome³.

Furthermore, the present study showed that renal AS score ≥ 1 , independently from the presence of HT, was associated with an increased risk of renal composite events. On this point, it is interesting that 88.3% of patients with normal-range BP had renal AS score ≥ 1 in the present study. Although renal AS might be associated with concurrent HT,^{5–8} it is sometimes found even in patients without HT¹⁹. Previous studies showed that renal AS was associated with aging²⁰, postprandial hyperglycemia and hyperinsulinemia²¹, low serum high-density lipoprotein cholesterol²², hyperuricemia^{23,24}, and chronic inflammation²⁵. In contrast, a BP $< 140/90$ mmHg is recommended to reduce ESRD and cardiovascular disease among people with diabetes in clinical practice¹. Lower BP targets might be considered for albuminuric patients with diabetes^{1,26}. Despite this BP control, the absolute residual risk for ESRD and cardiovascular disease is still extremely high in type 2 diabetes patients with diabetic nephropathy²⁷. The present results showed that the high prevalence of renal AS score ≥ 1 among patients with normal-range BP may be associated with some of the residual renal risk in type 2 diabetes patients with diabetic nephropathy.

Next, we showed that renal AS score ≥ 1 , independently from the presence of HT, was associated with an increased risk of cardiovascular events. Previous autopsy-based studies have reported that renal vascular changes were associated with atherosclerotic lesions in the coronary arteries, aorta and major cerebral vessels^{28–30}. In addition, kidney allograft biopsy samples have shown that carotid artery intima media thickness was correlated with the level of nephrosclerosis in kidney transplant donors³¹. These findings suggest that renal AS is not only

directly associated with renal outcomes, but also with cardiovascular outcomes.

The present study showed that the risk of renal composite events and cardiovascular events was the highest in both renal AS and HT. The cumulative incidence showed an association between HT and a greater increase in renal composite events and cardiovascular events in patients with renal AS score ≥ 1 , but not in patients with no renal AS. This finding might support the clinical importance of evaluating of renal AS in type 2 diabetes patients with diabetic nephropathy. The strength of the present study is that we directly evaluated renal AS status by renal biopsy for the assessment of its prognostic significance. However, pathological evaluation is not commonly applied in patients with diabetic nephropathy with a typical clinical course. Thus, whether the markers related to diabetic kidney lesions improve the predictive power when added to clinical findings is an important issue. Previous studies have reported a correlation between a high renal arterial resistive index (RRI) assessed by Doppler and advanced renal AS in chronic kidney disease patients, suggesting a role for as a severity marker^{32,33}. In addition, previous studies have reported that RRI was correlated with disease stage in patients with diabetic nephropathy³⁴ and organ damage severity (carotid intima-media thickness and ambulatory arterial stiffness index) in patients with HT³⁵, suggesting a role for RRI as a prognostic marker of renal and cardiovascular outcomes³³. Furthermore, brachial artery flow-mediated dilatation and renal small artery sclerosis have been reported to progress in parallel with chronic kidney disease progression³². However, an observational study of Japanese chronic kidney disease patients showed that vascular function indexes, such as central systolic BP, cardio-ankle vascular index and RRI, were not related to renal AS²². Further studies are required to clarify the surrogate markers of renal pathological changes in diabetic nephropathy.

There were some limitations in the present study. First, only a single measurement of BP at baseline was used. This might have caused the misclassification of study patients. Second, the treatment contents were not evaluated during the follow-up period. Third, there was likely an influence of bias through the limitation of participants with a renal biopsy. However, the long-term observation of 185 type 2 diabetes patients with biopsy-proven diabetic nephropathy is important for understanding the predictive effect of kidney lesions on clinical outcomes.

In conclusion, we showed that renal AS is associated with increasing risks for renal composite events and cardiovascular events in both normal-range BP and HT in type 2 diabetes patients with diabetic nephropathy. The risk of these events was the highest in both renal AS and HT. The high prevalence of advanced renal AS among normal-range BP as well as HT highlights the importance of taking renal AS status into consideration when trying to reduce the burden of renal and cardiovascular outcomes in type 2 diabetes patients with diabetic nephropathy.

ACKNOWLEDGMENT

This study was supported by JSPS KAKENHI Grant Number 17K08979 (MS).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

- American Diabetes Association. Standards of medical care in diabetes - 2018. *Diabetes Care* 2018; 41 (Suppl. 1): S86–S118.
- Tervaert TW, Mooyaart AL, Amann K, *et al.* Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol* 2010; 21: 556–563.
- Shimizu M, Furuichi K, Toyama T, *et al.* Long-term outcomes of Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy. *Diabetes Care* 2013; 36: 3655–3662.
- Shimizu M, Furuichi K, Yokoyama H, *et al.* Kidney lesions in diabetic patients with normoalbuminuric renal insufficiency. *Clin Exp Nephrol* 2014; 18: 305–312.
- Kubo M, Kiyohara Y, Kato I, *et al.* Risk factors for renal glomerular and vascular changes in an autopsy-based population survey: the Hisayama study. *Kidney Int* 2003; 63: 1508–1515.
- Ninomiya T, Kubo M, Doi Y, *et al.* Prehypertension increases the risk for renal arteriosclerosis in autopsies: the Hisayama Study. *J Am Soc Nephrol* 2007; 18: 2135–2142.
- Isobe S, Ohashi N, Ishigaki S, *et al.* Increased nocturnal blood pressure variability is associated with renal arteriolar hyalinosis in normotensive patients with IgA nephropathy. *Hypertens Res* 2017; 40: 921–926.
- Kopp JB. Rethinking hypertensive kidney disease: arterionephrosclerosis as a genetic, metabolic, and inflammatory disorder. *Curr Opin Nephrol Hypertens* 2013; 22: 266–272.
- The committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig* 2010; 1: 212–228.
- Matsuo S, Imai E, Horio M, *et al.* Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; 53: 982–992.
- Japanese Society of Hypertension Committee for Guidelines for the Management of Hypertension. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2014). *Hypertens Res* 2014; 37: 253–390.
- Gellman DD, Pirani CL, Soothill JF, *et al.* Diabetic nephropathy: a clinical and pathologic study based on renal biopsies. *Medicine (Baltimore)* 1959; 38: 321–367.
- Furuichi K, Yuzawa Y, Shimizu M, *et al.* Nationwide multicenter kidney biopsy study of Japanese patients with type 2 diabetes. *Nephrol Dial Transplant* 2018; 33: 138–148.
- Takazakura E, Nakamoto Y, Hayakawa H, *et al.* Onset and progression of diabetic glomerulosclerosis; a prospective study based on serial renal biopsies. *Diabetes* 1975; 24: 1–9.
- Levey AS, Inker LA, Matsushita K, *et al.* GFR decline as an end point for clinical trials in CKD: a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. *Am J Kidney Dis* 2014; 64: 821–835.
- Shimizu M, Furuichi K, Toyama T, *et al.* Decline in estimated glomerular filtration rate is associated with risk of end-stage renal disease in type 2 diabetes with macroalbuminuria: an observational study from JDNCS. *Clin Exp Nephrol* 2018; 22: 377–387.
- Hørlyck A, Gundersen HJ, Osterby R. The cortical distribution pattern of diabetic glomerulopathy. *Diabetologia* 1986; 29: 146–150.
- Bohle A, Wehrmann M, Bogenschütz O, *et al.* The pathogenesis of chronic renal failure in diabetic nephropathy. Investigation of 488 cases of diabetic glomerulosclerosis. *Pathol Res Pract* 1991; 187: 251–259.
- Wu J, Chen X, Xie Y, *et al.* Characteristics and risk factors of intrarenal arterial lesions in patients with IgA nephropathy. *Nephrol Dial Transplant* 2005; 20: 719–727.
- Hommos MS, Glassock RJ, Rule AD. Structural and functional changes in human kidneys with healthy aging. *J Am Soc Nephrol* 2017; 28: 2838–2844.
- Ike R, Honda K, Ishioka K, *et al.* Postprandial hyperglycemia and hyperinsulinemia associated with renal arterio-arteriosclerosis in chronic kidney disease. *Hypertens Res* 2010; 33: 499–504.
- Namikoshi T, Fujimoto S, Yorimitsu D, *et al.* Relationship between vascular function indexes, renal arteriosclerosis, and renal clinical outcomes in chronic kidney disease. *Nephrology* 2015; 20: 585–590.
- Kohagura K, Kochi M, Miyagi T, *et al.* An association between uric acid levels and renal arteriopathy in chronic kidney disease: a biopsy-based study. *Hypertens Res* 2013; 36: 43–49.
- Momoki K, Kataoka H, Moriyama T, *et al.* Hyperuricemia as a predictive marker for progression of nephrosclerosis: clinical assessment of prognostic factors in biopsy-proven arterial/arteriolar nephrosclerosis. *J Atheroscler Thromb* 2017; 24: 630–642.
- Miyagi T, Kohagura K, Ishiki T, *et al.* Interrelationship between brachial artery function and renal small artery sclerosis in chronic kidney disease. *Hypertens Res* 2014; 37: 863–869.
- Japanese Society of Nephrology. Evidence-based clinical practice guideline for CKD 2013. *Clin Exp Nephrol* 2014; 18: 346–423.
- Heerspink HJ, de Zeeuw D. The kidney in type 2 diabetes therapy. *Rev Diabet Stud* 2011; 8: 392–402.

28. Tracy RE, MacLean CJ, Reed DM, *et al.* Blood pressure, nephrosclerosis, and age autopsy findings from the Honolulu Heart Program. *Mod Pathol* 1988; 1: 420–427.
29. McGill HC Jr, Strong JP, Tracy RE, *et al.* Relation of a postmortem renal index of hypertension to atherosclerosis in youth. *Arterioscler Thromb Vasc Biol* 1995; 15: 2222–2228.
30. Burchfiel CM, Tracy RE, Chyou PH, *et al.* Cardiovascular risk factors and hyalinization of renal arterioles at autopsy. The Honolulu Heart Program. *Arterioscler Thromb Vasc Biol* 1997; 17: 760–768.
31. Erten S, Gungor O, Sen S, *et al.* Carotid artery intima media thickness (CA-IMT) is correlated with the level of nephrosclerosis in kidney transplant donors. *Nephrology* 2011; 16: 720–724.
32. Ikee R, Kobayashi S, Hemmi N, *et al.* Correlation between the resistive index by Doppler ultrasound and kidney function and histology. *Am J Kidney Dis* 2005; 46: 603–609.
33. Bigé N, Lévy PP, Callard P, *et al.* Renal arterial resistive index is associated with severe histological changes and poor renal outcome during chronic kidney disease. *BMC Nephrol* 2012; 13: 139.
34. Ishimura E, Nishizawa Y, Kawagishi T, *et al.* Intrarenal hemodynamic abnormalities in diabetic nephropathy measured by duplex Doppler sonography. *Kidney Int* 1997; 51: 1920–1927.
35. Florczak E, Januszewicz M, Januszewicz A, *et al.* Relationship between renal resistive index and early target organ damage in patients with never-treated essential hypertension. *Blood Press* 2009; 18: 55–61.