Relationship of renal morphology on 3-dimensional ultrasonography with renal pathologic findings and outcome in biopsy-proven nephropathy

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Abstract. Kidney morphology has been used to estimate renal functions. The present study investigated the usefulness of kidney length and renal parenchymal volume (RPV) measured using three-dimensional ultrasonography (3-D USG) in estimating renal pathological findings, and the outcome in patients with nephropathy who underwent renal biopsy. In this study, 94 adult patients who had native kidney biopsy with 3-D USG results were included. The mean kidney length and RPV were independent factors of, and positively correlated to the estimated glomerular filtration rate (eGFR). The mean kidney length and RPV had inverse associations with the percentage of global glomerulosclerosis. Higher mean RPV, other than longer kidney length, indicated a lower prevalence of tubular atrophy. During 63.3±19.3 months of follow-up, a mean RPV of <125 ml increased the risk of composite outcome by 4.287 fold (95% confidence interval, 1.133-16.227) as compared with a mean RPV of ≥125 ml (P=0.032). In conclusion, kidney size was inversely associated with certain nephronal damage and positively associated with eGFR in nephropathy. Furthermore, smaller RPVs predicted worse outcomes of nephropathy.

Introduction

Kidney morphology has been used to predict renal functions in healthy young adults (1). Several studies have also demonstrated the clinical implications of kidney morphology in pathological status. Kidney morphology is related to estimated glomerular filtration rate (eGFR) in patients with chronic kidney disease (CKD) (2-4), in obesity subjects (5), and in patients with nephrectomy due to renal tumor (6,7). Kidney morphology is also related to albuminuria (4) in CKD patients and related to the improvement of renal function after angioplasty in patients with renovascular hypertension (8). These studies suggested that kidney morphology could be closely related to eGFR or proteinuria when the kidney is in a pathological state, In addition, they suggested that the relationship between pathological findings derived from fresh tissues of living patients and kidney morphology could possibly contribute to the explanation of the relationship between kidney morphology and eGFR or between kidney morphology and proteinuria in patients with nephropathy.

Kidney volume and length were usually measured for kidney morphology. However, kidney volume estimated by using computed tomography (CT) predicted eGFR in healthy young adults, whereas kidney length was reported to be not related to eGFR (1). In patients with CKD, kidney volume is a more sensitive indicator of eGFR than kidney length (2). Kidney volume correlates with anthropometric parameters, particularly body surface area (BSA), whereas kidney length only has a weaker relationship with anthropometric parameters (3). Kidney volume is closely correlated to kidney weight (9,10), to which both glomerular number and size show a positive correlation (10). Kidney length shortens with age, while kidney volume is relatively stable (5,9,11). Thus, kidney volume may be a better indicator of eGFR in subjects with or without renal disease.

Contrast-enhanced CT and magnetic resonance imaging (MRI) are known to provide more accurate measurements of kidney volume than ultrasonography (USG) (4,6,12). However, these techniques are costly and have probable adverse effects in CKD patients due to contrast agents. USG has been improved in terms of both spatial and temporal resolutions to acquire three-dimensional (3-D) images. Three-dimensional USG estimates single kidney GFR in patients with nephrectomy (7) more accurately than two-dimensional USG (13). Renal parenchymal volume (RPV), including the renal cortex and medulla but excluding the renal sinus can be measured by using 3-D USG, repeatedly and non-invasively, at bedside (7), and could be used as an indicator of kidney volume.

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Therefore, we measured kidney length and RPV by using 3-D USG and investigated the efficacy of these parameters to estimate eGFR, proteinuria, renal pathological findings, and the outcomes in patients with nephropathy. We also investigated the relations among kidney morphology, renal pathological findings, and eGFR or proteinuria in patients with nephropathy.

Materials and methods

Patients. This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB no. B-1510/318-111) and was designed as a retrospective cohort study. None of the patients belonged to a vulnerable population or were subject to coercion. As this was a retrospective medical record-based study and the subjects were de-identified, the IRB waived the need for written consent from the patients. We included 99 adult patients who had a kidney biopsy for diagnosis of the cause of proteinuria, hematuria, and/or azotemia, which had persisted for more than 3 months, and had available 3-D USG results from March 2004 to June 2009. Fifty-three (53.5%) of the patients were male and the mean age was 47.3±14.5 years old (range, 24-84 years), whereas forty-six (46.5%) of the patients were female and the mean age was 43.5±18.8 years (range, 19-87 years). Among these patients, three who had a transplanted kidney biopsy, and two with fewer than 10 glomeruli found in the renal tissue biopsy were excluded. For all of 94 patients included in the analysis, we measured morphological parameters for both kidneys.

Measurements. All data were retrieved through the electronic medical record system, which was approved as a stage-7 hospital information system by the Healthcare Information and Management Systems Society. All the patients were admitted for 2-3 days and had their renal length and volume measured by using 3-D USG before renal biopsy. The patients' heights and weights were measured with the GL-310 weight and height measuring system (GL310; G-Tech International Co., Uijungbu, Kyungki-Do, Korea) on the day of admission. Blood pressures (BP) were measured on the right arm in the seated position by using an automated BP measuring device (HEM-7133; Omron, Kyoto, Japan). We adopted for analysis the morning BP on the second hospital day before renal biopsy. BSA was calculated as previously described (14). Diabetes mellitus (DM) was defined as the use of anti-diabetic medications, a serum fasting glucose level of ≥126 mg/dl or a glycosylated hemoglobin (HbA1c) level of ≥6.5%. Hypertension was defined as a systolic blood pressure of ≥140 mmHg, a diastolic blood pressure of ≥ 90 mmHg or the use of anti-hypertensive medication. Serum creatinine level was measured by using the modified alkaline picrate Jaffe kinetic method with an automatic analyzer (Toshiba 200FR; Toshiba Medical System Co., Tokyo, Japan). Serum creatinine levels were calibrated to an assay traceable by an isotope dilution mass spectrometry device (Modular P; Roche Diagnostics, Mannheim, Germany). As reported by the manufacturer, the lyophilized calibrator [Roche Calibrator for Automated Systems (CFAS), catalog no. 759350] was prepared with 3.0 ml of Milli-Q water. The

C.F.A.S. calibrator was traceable to reference material SRM 909b which was provided by the National Institute of Standards and Technology. The Modular P automatically performed a two-point calibration when there was a reagent lot number change and monitored control values to determine stability of the current calibration. eGFR was calculated by using the CKD-epidemiology collaboration (CKD-EPI) equation (15). On the second hospital day before renal biopsy, morning spot urine samples were collected to measure protein to creatinine ratio (UPCR) in the unit of g/g creatinine. We retrieved data on serum creatinine level, UPCR, and complete blood cell counts at admission for renal biopsy, and other data reported within 4 weeks before renal biopsy. We collected complete data for all the patients.

Measurement of renal morphological parameters for both kidneys was conducted with 3-D USG by using an iU-22 ultrasound unit (Philips iU-22; Philips Healthcare, Bothell, WA, USA). The transducer was positioned on the flank region while the patient was in a decubitus position. The maximal length of the kidney was measured in a long-axis view with a 2 to 5 MHz curved array transducer. After measurement of kidney length on the 2-D plane, the 3-D mode was activated and kidney volume data were acquired with a V2-6 broadband curved array transducer. Volume data acquisition scans for each kidney required 3 or 4 sec breath-holds. The acquired volume data were automatically stored and calculated as RPV, excluding volumes of the renal sinus, cysts, or tumors, and using a stacked contour tool installed in the ultrasonography unit (QLAB GI 3DQ Plug-in; Philips Healthcare). Both iU-22 ultrasound and ultrasonography unites were calibrated and manipulated by an experienced expert. The same radiologist measured the morphological parameters for all the patients. Provided that we did not measure the reference values of the kidney length and volume in subjects without renal disease, we used the value reported by Lee *et al* as the reference value for normal mean kidney length (10.71±0.74 cm) measured by using 2-D USG (16) and the value reported by Kim *et al* as the reference value for normal mean RPV measured by using 3-D USG for Koreans without CKD (175±20 ml) (13). We defined short kidney length if the length of both kidneys was <9.5 cm (approximately less than the mean value-2 x the standard deviation of length) and a small RPV if the mean volume for both kidneys was <125 ml (less than the mean value -2 x the standard deviation of volume). We also investigated the medication use before and after the pathological diagnosis. None of the patients received immunosuppressants before renal biopsy.

Pathological diagnosis. All biopsy results were evaluated by using appropriate standards for renal biopsy, including hematoxylin and eosin, periodic acid-Schiff, Masson trichrome, and periodic acid methenamine silver stainings for light microscopy (LM); immunofluorescence (IF) stainings using antibodies against IgA, IgG, IgM, C3, C1q, and kappa and lambda light chains; and electron microscopic examinations, as previously described (17). The pathological diagnosis was classified as either a nephritic or a non-nephritic lesion. One pathologist reviewed the pathologic findings. Pathological findings, which were graded semi-quantitatively, were graded from normal to severe (absence of lesion, focal mild changes:

Presence of the lesion in <25% of results, focal moderate changes: Presence of the lesion in 25-50% of results, focal marked or diffuse changes: Presence of the lesion in >50% of results). The patients were also grouped according to the presence or absence of a lesion.

Outcome. The primary outcome was the composite of death from any cause and/or progression to end stage renal disease (ESRD) that required renal replacement therapy after renal biopsy. Mortality data, available since 1992, were retrieved from the Korean National Statistical Office; and ESRD progression data, available since 1986, were retrieved from the Korean ESRD registry. We combined all of these data together.

Statistics. The data are shown as mean ± SD. All the analyses were conducted by using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). The correlations between eGFR, UPCR, or pathological findings and kidney length or volume were assessed by using the Pearson's coefficient. Multivariate analyses to detect factors independently related to continuous and binomial categorical variables, were performed by using linear and logistic regression analyses, respectively, adjusted for related factors. Differences in continuous variables were analyzed by using two-tailed, unpaired Student's t-tests or one-way analysis of variance according to the number of subgroups. By contrast, differences in categorical variables were analyze by using Chi-square tests or the Fisher's exact test according to the number of cases in each cell. We analyzed the cumulative incidence of the outcome between the patients categorized into groups according to a mean kidney length of 9.5 cm or the mean RPV of both kidneys of 125 ml, by using the Kaplan-Meier analysis. Incidence rates were compared by using the log-rank test. The receiver-operator curve (ROC) was also used to identify the cutoff value of mean kidney length or the mean RPV of both kidneys in our cohort and Youden's index was applied for the selection of the best cutoff value. We also analyzed the cumulative incidence of the outcome between the patients categorized into groups according to the best cutoff value by using the Kaplan-Meier analysis. To determine whether renal morphological data were independently related to the incidence of the outcome, we used the Cox hazard proportional analysis. Two-sided P-values were reported with 0.05 selected as the level of statistical significance.

Results

Patient characteristics. Forty-two of the patients were men (44.7%), and the mean age was 45.5±16.4 years (range, 19-84 years; Table I). Of the patients, 56.4% (53/94), 17.0% (16/94), and 26.6% (25/94) had eGFRs of ≥90, 60-89, <60 ml/(min·1.73 m^2), respectively. The mean UPCR was 3.88 g/g of Cr, and 43.6% (41/94) of the patients had an UPCR ≥3.00 g/g Cr. The diagnosis was nephritic in 50 patients and 44 non-nephritic lesion in 44 patients. The most common pathological diagnosis was IgA nephropathy (36.2 %), followed by minimal change lesion (9.6%) and membranous nephropathy (8.5%; Table I). Pathological findings are described in Table II. The mean length and parenchymal volume of both kidneys were 10.6±0.9 cm and 138.3±34.6 ml, respectively. No significant difference in mean kidney length or RPV was found

Table I. Basal characteristics of the patient with renal biopsy.

Parameters	Values
Age (years)	45.5±16.4
Male	42 (44.7)
Height (cm)	163±9
Weight (kg)	63.3±13
BMI (kg/m^2)[a]	23.8±3.9
BSA (m^2)[a]	1.68±0.2
SBP (mmHg)[a]	121±17
DBP (mmHg)[a]	69±12
Creatinine (mg/dl)	1.43±1.78
eGFR (ml/min/1.73 m^2)[a]	84.8±38.9
UPCR (g/g cr)[a]	3.88±5.33
Diabetes mellitus	14 (14.9)
Hypertension	62 (66)
Total kidney	
Mean length (cm)[a]	10.6±0.9
Mean RPV (ml)[a]	138.3±34.6
Left kidney	
Length (cm)[a]	10.6±1.1
RPV (ml)[a]	141.8±37.1
Right kidney	
Length (cm)[a]	10.6±0.9
RPV (ml)[a]	134.7±35.8
Urinalysis	
Albumin negative	15 (16)
Trace	5 (5.3)
1+	5 (2.3)
2+	19 (20.2)
≥3+	50 (53.2)
RBC[a]	
<5/HPF[a]	31 (33)
5-9/HPF	12 (12.8)
≥10/HPF	51 (54.3)
WBC[a]	
<5/HPF	72 (76.6)
5-9/HPF	10 (10.6)
≥10/HPF	12 (12.8)
Immunosuppressant[a]	35 (37.2)
Steroid	33 (35.1)
Cyclophosphamide	14 (14.9)
Others[a]	17 (18.1)
Pathologic diagnosis[a]	
Non-nephritic	
AIN	2 (2.1)
Alport's syndrome	1 (1.1)
Amyloidosis	3 (3.2)
C1q nephropathy	3 (3.2)
CIN	3 (3.2)
Diabetic nephropathy	3 (3.2)
FSGS	6 (6.4)
Hypertensive	2 (2.1)
MCD	9 (9.6)

Table I. Continued.

Parameters	Values
Myeloma kidney	1 (1.1)
MN	8 (8.5)
Thin membrane disease	3 (3.2)
Nephritic	
Crescentic	
GN	3 (3.2)
DPLN	3 (3.2)
HSN	1 (1.1)
IgA nephritis	34 (36.2)
MPGN	2 (2.1)
Non-specific GN	6 (6.4)
PSGN	1 (1.1)

Values are presented as the mean ± standard deviation or the number of patients with the corresponding percentage. [a]BMI, body mass index; SBP, systolic blood pressure; BSA, body surface area; DBP, diastolic blood pressure; eGFR, calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using isotope-dilution mass spectrometry (IDMS) traceable creatinine; UPCR, urine protein to creatinine ratio in g/g creatinine unit, length: The longest length of kidney; RPV, renal parenchymal volume, Mean length: Mean length of both kidneys; Mean RPV, mean parenchymal volume of both kidneys; RBC, red blood cell; HPF, high power-field under 400-fold magnification of light microscopy; WBC, white blood cell, immunosuppressant: Medication after renal biopsy during follow-up period, including steroid, cyclophosphamide, mycophenolate, cyclosporine, tacrolimus, or azathioprine, others: Immunosuppressant including mycophenolate, cyclosporine, tacrolimus, or azathioprine; AIN, acute interstitial nephritis; CIN, chronic interstitial nephritis; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MN, membranous nephropathy; GN, glomerulonephritis; DPLN, diffuse proliferative lupus nephritis, lupus nephritis class IV defined by International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification; HSN, Henoch-Schölein nephritis; MPGN, membranoproliferative nephritis; PSGN, post-streptococcal glomerulonephritis.

between the left and right kidneys. The mean RPV showed significant correlations with weight, height, body mass index (BMI), and BSA in 94 subjects. The mean kidney length also showed significant correlations with weight, height, and BMI, but weaker correlations compared with those of the mean RPV (Table III). In the multiple linear regression analysis, height was the only independent factor related to mean kidney length (B=0.026, P<0.001); and BSA was related to mean RPV (B=77.750, P<0.001).

Renal function, kidney morphology and pathologic findings. We analyzed the relationship between kidney morphology and renal function by using multiple linear regression analysis adjusted for clinical parameters (Table IV). Both mean kidney length and RPV were identified as independent factors with positive correlations with eGFR level. However, they were not related to UPCR. The pathological findings related to eGFR were percent of global glomerulosclerosis (P=0.037) and grade of interstitial inflammation (P<0.001) in the multiple

Table II. Pathologic findings of renal biopsy.

Pathologic findings	Values
No. of glomerulus	48.3±22.6
Global glomerulosclerosis (%)	17.1±22.8
Segmental glomerulosclerosis (%)	5.3±9.7
Glomerular crescent (%)	0.2±0.4
Glomerular necrosis (%)	0.0±0.2
Glomerular ischemia (%)	0.1±0.3
Glomerular hypertrophy (%)	
None	59.6
Mild	31.9
Moderate	7.4
Severe	1.1
Glomerular cellularity (%)	
None	29.8
Mild	55.3
Moderate	10.6
Severe	4.3
Mesangial proliferation (%)	
None	27.7
Mild	17
Moderate	41.5
Severe	13.8
Interstitial fibrosis (%)	
None	26.6
Mild	50
Moderate	13.8
Severe	9.6
Interstitial inflammation (%)	
None	26.6
Mild	48.9
Moderate	11.7
Severe	12.8
Tubular atrophy (%)	
None	24.5
Mild	50
Moderate	12.8
Severe	12.8
Vascular lesion (%)	
Absence	60.6
Presence	39.4

Values are presented as the mean ± standard deviation or percentage. One pathologist counted the percentages of glomeruli with global sclerosis, segmental sclerosis, crescent, ischemic changes, or necrosis, and assessed the renal changes of glomerular hypertrophy, glomerular cellularity, mesangial proliferation, interstitial fibrosis, interstitial inflammation, and tubular atrophy, semi-quantitatively. We scored from normal to severe form (absence of lesion, focal mild changes: Presence of the lesion in <25% of results, focal moderate changes: Presence of the lesion in 25-50% of results, focal marked or diffuses changes: Presence of the lesion in >50% of results). We assessed the arteriolar hyalinosis and arteriosclerosis defined as non-hyaline thickening of the vascular wall with reduction of the lumen. We grouped the arteriolar lesion as presence or absence.

Table III. Correlation between anthropometric parameters and kidney size.

Parameter	Mean kidney length		Mean RPV	
	r[a]	P-value	r[a]	P-value
Age	-0.043	0.681	-0.073	0.485
Gender	-0.005	0.962	0.182	0.080
Weight	0.249	0.016	0.430	<0.001
Height	0.257	0.013	0.366	<0.001
BMI	0.274	0.007	0.455	<0.001
BSA	0.140	0.179	0.285	0.005

[a]r, Pearson's correlation coefficient; RPV, renal parenchymal volume; BMI, body mass index; BSA, body surface area.

Table IV. The correlation between morphometric measurements of kidney and eGFR or proteinuria.

Measurement	B	t	95% CI for B		P-value
For eGFR[a]					
Mean kidney length (cm)	11.601	4.082	5.941	17.261	<0.001
Mean RPV (10 ml)	1.895	2.250	0.022	0.357	0.027
For UPCR[a]					
Mean kidney length (cm)	uc	-1.042	-0.110	0.848	0.300
Mean RPV (10 ml)	uc	1.722	0.181	0.905	0.089

[a]eGFR, calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using isotope-dilution mass spectrometry (IDMS) traceable creatinine; eGFR, estimated glomerular filtration rate. UPCR, urine protein to creatinine ratio in g/g creatinine unit; RPV, renal parenchymal volume; uc, unable to calculate; CI, confidence interval. Multivariate linear regression analysis was applied to reveal the correlation between eGFR and morphometric measurement, adjusted for age, sex, presence of diabetes mellitus and hypertension, systolic blood pressure, levels of hemoglobin, serum C-reactive protein, phosphorus, uric acid, and presence of hematuria. To reveal the correlation between UPCR and morphometric measurement, it was adjusted for age, sex, presence of diabetes mellitus, levels of serum albumin, bilirubin, cholesterol, glucose, phosphorus, and uric acid, which were related factors to eGFR or UPCR.

linear regression analysis adjusted for interstitial fibrosis, tubular atrophy, and pathologic diagnosis. For the UPCR, the only independent pathological factor was percent of global glomerulosclerosis (P=0.050) adjusted for pathological diagnosis.

Mean kidney length and RPV were inversely related to percent of global glomerulosclerosis, grades of interstitial fibrosis and inflammation, and tubular atrophy (Table V). For each pathological finding, the multivariate analysis revealed that mean kidney length and RPV had inverse relationships with percent of global glomerulosclerosis (Table VI). These

Table V. The correlation coefficients between renal morphology and pathologic findings.

Characteristic	Mean kidney length (cm)	Mean RPV (ml)
Glomerular hypertrophy (grade)	0.065	0.109
Global glomerulosclerosis (%)	-0.489[a]	-0.392[a]
Segmental glomerulosclerosis (%)	-0.018	-0.106
Glomerular crescent (%)	0.151	0.086
Glomerular necrosis (%)	0.035	-0.064
Glomerular ischemia (%)	-0.028	0.030
Glomerular cellularity (grade)	0.104	-0.044
Mesangial proliferation (grade)	0.084	-0.030
Interstitial fibrosis (grade)	-0.340[b]	-0.269[b]
Interstitial inflammation (grade)	-0.318[b]	-0.302[b]
Tubular atrophy (grade)	-0.341[b]	-0.319[b]
Vascular lesion (presence or absence)	0.070	0.015

[a]P<0.001, [b]P<0.01; RPV, renal parenchymal volume. One pathologist counted the percentage of glomeruli with global sclerosis, segmental sclerosis, crescent, ischemic changes, or necrosis, and assessed the renal changes of glomerular hypertrophy, glomerular cellularity, mesangial proliferation, interstitial fibrosis, interstitial inflammation, and tubular atrophy, semi-quantitatively. We scored the findings from normal to severe (absence of the lesion, focal mild changes: Less than 25% are affected by the lesion, focal moderate changes: 25-50% are affected, focal marked or diffuse changes: 50% or more are affected). We assessed the arteriolar hyalinosis and arteriosclerosis defined as non-hyaline thickening of the vascular wall with reduction of the lumen. We grouped the arteriolar lesion as presence or absence.

relationships were also evident in patients with or without nephritis (data not shown). No collinearity was found between eGFR, another independent factor of global glomerulosclerosis, and mean kidney length or RPV (variance inflation factor=1.108 and 1.020, respectively) when we estimated percent of global glomerulosclerosis by using multivariate analysis. Higher mean RPV, but not kidney length, indicated lower prevalence of tubular atrophy in multivariate analysis.

Kidney morphology and the outcome. During 63.3±19.3 months of follow-up, the disease progressed to ESRD in five patients, all of whom were alive at the end of the follow-up period. Six deaths, including two cases of cancers, one case of chronic obstructive lung disease, one case of glomerulonephritis, one case of chronic renal failure, and one case of accident were observed. The ROC analysis indicated that best cutoff point of mean kidney length and mean RPV was 9.4 cm and 120.7 ml, respectively. Patients with small mean RPVs of <125 ml tended to have a higher incidence rate of ESRD or mortality (Fig. 1), and showed a higher rate of composite outcome by Fisher exact test and log-rank test (Figs. 1 and 2). Patients with small mean RPVs of <120.7 ml displayed a higher incidence rate of ESRD (Fig. 1) and composite outcome by Fisher's exact test and log-rank test (Figs. 1 and 2). With the Cox proportional hazard model, the mean RPV of <125 ml

Table VI. Multivariate analysis for factors related to pathologic findings of renal biopsy.

Pathology	Glomerular sclerosis (%)		Interstitial fibrosis (presence)		Interstitial inflammation (presence)		Tubular atrophy (presence)	
Parameter	t-value	P-value	OR	P-value	OR	P-value	OR	P-value
Model 1[a]								
Mean kidney length	-3.819	<0.001	uc	0.135	uc	0.271	uc	0.181
Mean RPV	-3.939	<0.001	uc	0.087	uc	0.364	0.983	0.026
Model 2[b]								
Mean kidney length	-3.600	0.001	uc	0.682	uc	0.984	uc	0.938
Mean RPV	-3.506	0.001	uc	0.267	uc	0.540	0.977	0.016

RPV, renal parenchymal volume; OR, Odds ratio; uc, unable to calculate. [a]Model 1, all analyses were adjusted for age, sex, presence of diabetes mellitus and hypertension, eGFR, and UPCR. [b]Model 2, multivariate analyses were analyzed with adjustment for the factors related to each pathologic finding. For the percent of glomerular sclerosis, the multiple linear regression analysis was adjusted for the presence of diabetes mellitus, serum levels of albumin, complement C4, cholesterol, eGFR, hemoglobin, and uric acid, and morphologic data measured by ultrasonography. For the presence of interstitial fibrosis, the multiple logistic regression analysis was adjusted for the presence of hypertension, serum bilirubin more than 0.6 mg/dl, serum level of complement C4, cholesterol, eGFR, hemoglobin, protein, uric acid, presence of pyuria, and morphologic data measured by ultrasonography, and, for the presence of interstitial inflammation, adjusted for age, presence of hypertension, serum bilirubin more than 0.6 mg/dl, serum levels of complement C4, cholesterol, C-reactive protein, eGFR, GOT, hemoglobin, protein, uric acid, level of WBC, presence of hematuria and albuminuria ≥3 + by a dipstick test, and morphologic data measured by ultrasonography, and, for the presence of tubular atrophy, adjusted for age, presence of hypertension, serum bilirubin more than 0.6 mg/dl, serum levels of complement C4, cholesterol, C-reactive protein, eGFR, GOT, hemoglobin, protein, uric acid, level of WBC, and morphologic data measured by ultrasonography.
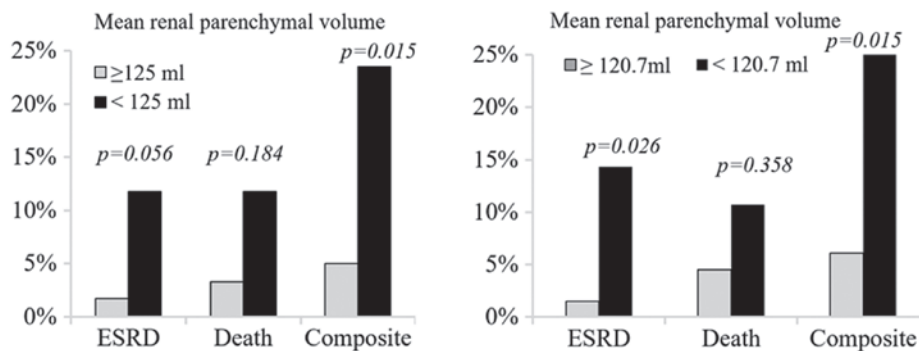

Figure 1. The incidence of outcomes according to mean renal parenchymal volume. The P-value was estimated by using the Fisher's exact test. Sixty patients had a mean parenchymal volume of ≥125 ml, and 34 patients had a mean parenchymal volume of <125 ml whereas, sixty-six patients had a mean parenchymal volume of ≥120.7 ml, and 28 patients had a mean parenchymal volume of <120.7 ml.

increased the risk of composite outcome by 4.287-fold [95% confidence interval (CI): 1.133-16.227] as compared with the mean RPV ≥125 ml (P=0.032; Table VII), whereas, the mean RPV of <120.7 ml increased the risk of composite outcome by 3.395-fold (95% CI: 0.985-11.6) as compared with the mean RPV ≥120.7 ml (P=0.053; Table VIII). However. In a fully-adjusted model which adjusted with mean RPV categorized by 125 ml and 120.7 ml, the mean RPV of <125 ml, other than the mean RPV of <120.7 ml (P=0.882), was a risk factor for the composite outcome (hazard ratio: 4.287, 95% CI: 1.133-16.227, P=0.032). Interaction terms were created between mean RPV and other risk factors (eGFR, grade of interstitial fibrosis, tubular atrophy, and percent of glomerulosclerosis in renal biopsy findings) to identify significant interactions. The only interaction term that was significantly associated with the composite outcome was mean RPV x percent of glomerulosclerosis (P=0.023). Another factor independently associated with the composite outcome was eGFR (P=0.002).

Discussion

In this study, kidney morphological parameters were obtained by using 3-D USG, which was more accurate and robust than 2-D USG, and cheaper and less harmful than MRI. 3-D volumetry has lower mean absolute deviation in related to the gold-standard measurements (the fluid displacement method provided the 'gold standard') in assessing the volume of human

Table VII. The relationship between morphometric measurements of the kidney on the outcome of patients with nephropathy (reference values were obtained from subjects without renal disease).

Morphometric variables[a]	Model 1[a]		Model 2[b]				
	B	P-value	B	RR	95% CI of RR		P-value
Mean kidney length	uc	0.381	nd	nd	nd	nd	nd
Group of length (<9.5 cm)	uc	0.057	nd	nd	nd	nd	nd
Mean RPV	uc	0.204	nd	nd	nd	nd	nd
Group of RPV (<125 ml)	1.623	0.017	1.456	4.287	1.133	16.227	0.032

RR, relative risk; RPV, renal parenchymal volume; uc, unable to calculate; nd, not done; CI, confidence interval. [a]Model 1, Cox's proportional hazard model for the outcome with single morphometric variable. [b]Model 2, Cox's proportional hazard model for the outcome with each significant variable in model 1 and the other related factors, such as estimated glomerular filtration rate, hemoglobin, presence of hematuria and proteinuria ≥3 + by a dipstick test, grade of interstitial fibrosis, tubular atrophy and percent of glomerulosclerosis on renal biopsy findings, which were univariate factors to the outcome, age, and sex. Group of length: Patients grouped by the criterion of mean length of both kidneys 9.5 cm, Group of RPV: Patients grouped by the criterion of mean parenchymal volume of both kidneys 125 ml.

Table VIII. The relationship between morphometric measurements of the kidney on the outcome of patients with nephropathy (optimal cutoff values from ROC-curve analysis were used as reference values).

Morphometric variables[a]	Model 1[a]		Model 2[b]				
	B	P-value	B	RR	95% CI of RR		P-value
Mean kidney length	uc	0.381	nd	nd	nd	nd	nd
Group of length (<9.4 cm)	uc	0.047	uc	uc	uc	uc	0.216
Mean RPV	uc	0.204	nd	nd	nd	nd	nd
Group of RPV (<125.7 ml)	1.483	0.018	1.222	3.395	0.985	11.6	0.053

RR, relative risk; RPV, renal parenchymal volume; uc, unable to calculate; nd, not done. [a]Model 1, Cox's proportional hazard model for the outcome with single morphometric variable. [b]Model 2, Cox's proportional hazard model for the outcome with each significant variable in model 1 and the other related factors, such as estimated glomerular filtration rate, hemoglobin, presence of hematuria and proteinuria ≥3 + by a dipstick test, grade of interstitial fibrosis, tubular atrophy and percent of glomerulosclerosis on renal biopsy findings, which were univariate factors to the outcome, age, and sex. Group of length: Patients grouped by the criterion of mean length of both kidneys 9.4 cm, Group of RPV: Patients grouped by the criterion of mean parenchymal volume of both kidneys 120.7 ml.
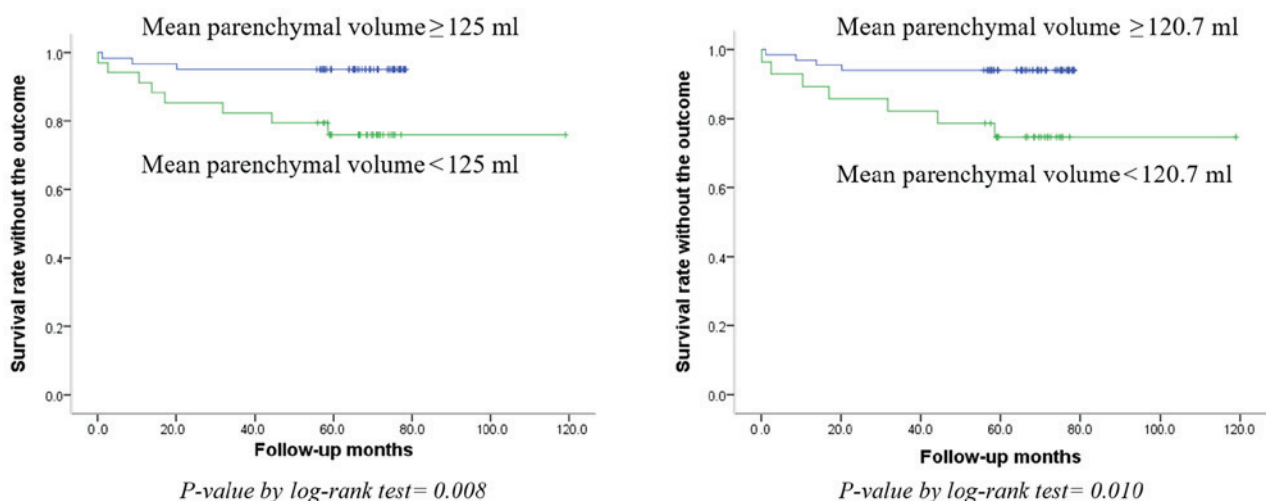

Figure 2. The results of the survival analysis of the outcomes according to the mean renal parenchymal volume of both kidneys. Patients with small mean RPVs of <125 ml showed a higher incidence rate composite outcome by log-rank test as compared with patients with small mean RPVs of ≥125 ml. Patients with small mean RPVs of <120.7 ml displayed a higher incidence rate composite outcome by log-rank test as compared with patients with small mean RPVs of ≥120.7 ml. RPV, renal parenchymal volume.

cadaver kidneys as compared with 2-D images, and also showed a satisfactory concordance correlation to gold-standard measurement (18). Volume measurements obtained from MRI with the voxel-count method showed little deviation from the true renal volume (gold-standard) (19). Kidney volume measured by 3-D USG and MRI yielded a good correlation (r=0.82, P<0.001) and the accuracy of 3-D USG was 91.6±8.4% in relation to MRI volumes of human kidneys (20). Therefore, 3-D USG performs high accuracy in renal volume estimation for clinical applications. Kidney size, particularly RPV, was inversely related to certain nephronal damage presenting as global glomerulosclerosis and tubular atrophy, whereas percent of global glomerulosclerosis (P=0.037) was inversely related to eGFR. This could be a pathophysiological mechanism to explain a positive relationship between renal morphology and eGFR, regardless of age, sex, and anthropometric characteristics. Furthermore, we found that smaller RPVs would predict worse outcomes in nephropathy.

Renal mass or volume was supposed to decline with age just as glomerular number and volume would (10). However, kidney mass or volume does not change until 50 or 60 years of age (9,11,12), and kidney mass and number of glomeruli decline with age after 60 years of age. As a result, this decline cannot be entirely explained by age-related glomerulosclerosis in subjects without renal disease (21). In this study, kidney length and RPV were not related to age because relatively young patients with mean glomerulosclerosis rates of around 20% due to nephropathy were included. Kidney volume best correlated with BSA, whereas kidney length correlated with height in healthy subjects (22). This correlation was also evident in patients with nephropathy.

We discovered that RPV was a useful parameter for estimating eGFR in patients with nephropathy and suggest that nephron damage such as glomerulosclerosis is a possible pathophysiological mechanism to explain the link between eGFR and RPV in patients with nephropathy. In autopsy cases without a history of renal disease, mean glomerular volume and number of glomeruli increased with kidney weight, and both matched well with kidney volume (10). Kidney volume was associated with BSA (2,5,10,11,21,23), even after adjustment for the influence of age. Volume of glomeruli was correlated to BSA, whereas number of glomeruli was not (10,24). Other factors need to be considered for the change in glomeruli volume. were the environmental factors and gene-environment interactions (24). Age and sex-matched African Americans and native West Africans in Senegal, who are presumed to have the same ancestry, have similar total nephron number per kidney. However, mean glomerular volume was greater in African Americans, which could not be completely accounted for differences in body size (25). Larger glomerular size was observed in African Americans who had the same range of body size as the Senegalese (24). Furthermore, an increase in glomerular volume was evident only in African Americans (24). High dietary protein intake also contributed to the enlargement of glomerular volume (26). The glomerular volume in subjects with the same number of glomeruli may change owing to genetic and environmental factors, high dietary protein intake and others. Researchers have proved the relationship between glomerular volume and pathological findings (21,25). Mean glomerular volume was increased in IgA

nephropathy with more severe glomerulosclerosis and tubular atrophy, and was estimated based on BMI and glomerular density (number of non-sclerotic glomeruli per renal cortical area in the biopsy samples) in the IgA nephropathy and non-glomerulonephritis groups (25). Hypertension and CKD were associated with greater mean glomerular volume (21,27). However, the kidney volumes in patients with renal disease may not increase. Although the remaining glomeruli may be enlarged to satisfy the metabolic demands, some nephrons may be damaged and lost. Therefore, glomerular volume may change due to metabolic demands, genetic-environmental interactions, and pathological injuries. RPV in terms of kidney volume may be reflected by glomerular volume and numbers of glomeruli. Previous studies did not investigate changes of kidney volume based on changes in glomerular volume and number of glomeruli, and changes in glomerular volume and number of glomeruli may be due to pathological harm in patients with renal disease. Thus, we investigated the efficacy of kidney volume on estimating pathological findings and outcomes in kidney diseases. In our study, pathologic changes of nephrons were used as link between kidney morphology and eGFR, and between kidney morphology and outcomes.

In patients with renal diseases, some nephrons may be damaged and lose function. Single-nephron glomerular hyperfiltration in remnant glomeruli due to functional nephron loss would lead to progressive glomerular hypertrophy and glomerulosclerosis (28,29). An inverse relationship between RPV and percent of global glomerulosclerosis was also investigated in our study. In patients with atherosclerotic renal artery stenosis, a higher ratio of RPV to GFR would guarantee a better residual renal function after revascularization, which consequently implied RPV as an indicator of 'hibernating' kidney under ischemic condition (12). The relationship between RPV at renal biopsy and the composite outcome in this study could be explained in a similar manner.

This study had limitations. First, it did not have a prospective design and therefore might have selection and data biases. However, the UPCR, serum creatinine, and urinalysis data were collected at renal biopsy in all the patients, and all the data analyzed were complete. The same radiologist and pathologist were involved throughout the study period. However, the data were collected over a period of 5 years and intra-person bias might be present within the data. Secondly, we did not measure kidney morphological parameters in a healthy population by using 3-D USG and did not collect nationwide data on normal range of RPV and kidney length for Koreans. We adopted the RPV and kidney length USG data for a population without renal diseases reported by other investigators and used these as reference for evaluating an exposed group to evaluate the risk of the study outcome. The data would have selection or investigator bias. In order to ensure the validity of our results, we also use a receiver-operator curve to identify the best cut off (mean RPV, 120.7 ml) in our cohort and used this cutoff for the Kaplan-Meier and Cox regression analysis. However, the results of Cox regression analysis also indicated that the mean RPV of <125 ml, other than the mean RPV of <120.7 ml (P=0.882), was a risk factor for the composite outcome (Hazard ratio: 4.287, 95% CI: 1.133-16.227, P=0.032) in a fully-adjusted model. Third, diabetic kidney disease and amyloidosis may not show atrophy even under eGFR decline. Thus, 125 ml RPV

may not be suitable as a reference value for evaluating the risk of outcome in subjects with diabetic and amyloidosis nephropathy. Three patients had diabetic nephropathy and another 3 had amyloidosis in our study. The mean RPV of <125 ml increased the risk of composite outcome by 4.287-fold (95% CI: 1.133-16.227) compared with the mean RPV of ≥125 ml. By contrast, the mean RPV of <125 ml increased the risk of composite outcome by 4.493-fold (95% CI: 0.901-22.406) as compared with the mean RPV of ≥125 ml after exclusion of diabetic and amyloidosis nephropathy. In the 3 diabetic or amyloidosis nephropathy, we observed a trend of higher RPV associated with longer follow-up duration. However, the cases of diabetic and amyloidosis nephropathy were inadequate to analyze the relationship between RPV and the outcome in diabetic and amyloidosis nephropathy. In conclusion, our study shows that smaller RPVs would predict worse outcomes in nephropathy. Further research on diabetic and amyloidosis nephropathy is need. Finally, we did not examine 3-D USG data of patients who underwent renal biopsy within 3 months after onset of symptoms by history-taking to exclude acute change of kidney morphology due to interstitial edema, and water and sodium retention.

In conclusion, kidney morphology is inversely related to nephronal damage and positively related to eGFR in nephropathy diagnosed by using renal biopsy. In the future, whether RPV is an important indicator to guide decision-making during treatment of nephropathies and to estimate renal outcomes of each type of nephropathy should be investigated.

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