Original Article

JVC Journal of Veterinary Clinics

pISSN 1598-298X • eISSN 2384-0749
J Vet Clin 2023;40:399-407
https://doi.org/10.17555/jvc.2023.40.6.399

Check for updates

Evaluation of Albumin Creatinine Ratio as an Early Urinary Biomarker for Chronic Kidney Disease in Dogs

Hyun-Min Kang[1]
Heyong-Seok Kim[2]
Min-Hee Kang[3]
Jong-Won Kim[2]
Dong-Jae Kang[1]
Woong-Bin Ro[4]
Doo-Won Song[5]
Ga-Won Lee[6]
Hee-Myung Park[1,*]
Hwi-Yool Kim[7,*]

[1]Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 05029, Korea
[2]Petobio, Inc., Hanam 12982, Korea
[3]Department of Bio-Animal Care, Jangan University, Hwaseong 18331, Korea
[4]Department of Veterinary Emergency and Critical Care Medicine, College of Veterinary Medicine, Chonnam National University, Gwangju 61186, Korea
[5]Shine Animal Medical Center, Seoul 05550, Korea
[6]Department of Companion Animal Industry, College of Health and Welfare, Dongshin University, Naju 58245, Korea
[7]Laboratory of Veterinary Surgery, College of Veterinary Medicine, Konkuk University, Seoul 05029, Korea
*Correspondence: parkhee@konkuk.ac.kr (Hee-Myung Park), kykim@konkuk.ac.kr (Hwi-Yool Kim)

ORCID
Hyun-Min Kang: https://orcid.org/0000-0003-1914-8112
Heyong-Seok Kim: https://orcid.org/0000-0003-1089-1460
Min-Hee Kang: https://orcid.org/0000-0001-7304-5064
Jong-Won Kim: https://orcid.org/0000-0002-4999-980X
Dong-Jae Kang: https://orcid.org/0000-0001-9965-6445
Woong-Bin Ro: https://orcid.org/0000-0001-7123-8436
Doo-Won Song: https://orcid.org/0000-0003-2596-9785
Ga-Won Lee: https://orcid.org/0000-0003-3299-898X
Hee-Myung Park: https://orcid.org/0000-0002-7254-9524
Hwi-Yool Kim: https://orcid.org/0000-0001-6237-9958

Abstract Chronic kidney disease (CKD) occurs in more than 15% of the dogs over 10 years of age and causes irreversible renal function deterioration. Therefore, it is important to diagnose CKD early and treat the disease properly. The purpose of this study aimed to to evaluate the clinical utility of urine albumin/creatinine ratio (ACR) using POC (point-of-care) device as an early detection urinary biomarker in CKD dogs and to confirm the correlation between ACR and other known CKD biomarkers. Urine and serum samples were obtained from 50 healthy dogs and 50 dogs with CKD. Serum blood urea nitrogen (BUN), creatinine, and symmetric dimethylarginine (SDMA) concentrations, and urine protein creatinine ratio (UPC) were measured. Urine specific gravity (USG) was evaluated using refractometer, and ACR was measured using an i-SENS A1Care analyzer. The ACR values of dogs with CKD were significantly different from those of healthy dogs ($p < 0.001$), as with other renal biomarkers. ACR showed significant differences between healthy dogs and dogs with CKD at every IRIS stage ($p < 0.005$), whereas no significant differences were observed between dogs with CKD IRIS stage I and healthy dogs with UPC. There are significant positive correlation between ACR and BUN ($r = 0.611$, $p < 0.001$), creatinine ($r = 0.788$, $p < 0.001$), SDMA ($r = 0.747$, $p < 0.001$), and UPC ($r = 0.784$, $p < 0.001$), and significant negative correlation between ACR and USG ($r = –0.700$, $p < 0.001$). In receiver operator characteristic curve analysis, the area under the curve (AUC) was 0.982 (95% CI 0.963-1.000, $p < 0.001$), with an optimal cut-off value of 64.20 mg/g (94% sensitivity and 94% specificity). Thus, ACR is a useful urinary biomarker for the early diagnosis of proteinuria in CKD and combined use of ACR and other renal biomarkers may be helpful for early diagnosis and prevention of CKD in dogs.

Key words canine, CKD biomarker, urine albumin creatinine ratio.

Received July 27, 2023 / Revised September 28, 2023 / Accepted November 7, 2023

Introduction

Chronic kidney disease (CKD) occurs in 1% of dogs, and its incidence increases by more than 15% in dogs over 10 years of age (3). CKD is considered one of the main causes of death in elderly dogs in today's scenario of increased life expectancy (2).

It causes irreversible renal function deterioration, and is associated of various health problems, such as uremia, electrolyte imbalance, metabolic acidosis, non-regenerative anemia, proteinuria, and hypertension (15).

As nephrons lose their function during CKD, the heavy load on the remaining functional nephrons causes an increase in the blood flow and glomerular filtration rate, which leads to enlargement and thickening of the glomerulus. Glomerular lesions such as glomerular sclerosis may eventually occur, resulting in proteinuria. Therefore, persistent proteinuria can be an indication of renal dysfunction (6), and is associated with an increased mortality rate in dogs with CKD (8). Attenuation of proteinuria increases the survival time of dogs with CKD (10).

The prognosis of CKD is associated with the severity of the disease, and the mean survival time of CKD dogs decreases with disease progression (16). Therefore, it is important to diagnose CKD early and provide appropriate treatment (5,11).

Currently, in veterinary medicine, circulating serum blood urea nitrogen (BUN), creatinine, symmetric dimethylarginine (SDMA), urine protein creatinine ratio (UPC), and urine specific gravity (USG) are the known renal biomarkers in dogs for representing glomerular function. Serum creatinine levels are not altered until 75% of the total nephron function is lost (5). Therefore, further research on various biomarkers is required. In a recent study, the values of urinary albumin/creatinine, urinary retinol-binding protein (RBP)/creatinine, and urinary N-acetyl-b-D-glucosaminidase (NAG)/creatinine were critically elevated in dogs with CKD, and a strong association with BUN, creatinine was found (18).

Microalbuminuria, which can be confirmed by the urine albumin/creatinine ratio (ACR), refers to a state in which urine albumin excretion is increased but can't be measured using conventional methods, and implies impaired glomerular filtration (13,19). In human medicine, ACR, called urinary albumin-to-creatinine ratio (UAC) in veterinary medicine, is the first biomarker used to detect elevated protein levels in the urine when GFR decreases (7). However, the amount of urine albumin can be affected by the posture of the patient, hydration state, physical activity, blood pressure, stress, infection, and pregnancy (12).

In veterinary medicine, ACR is not routinely used to diagnose CKD as no point-of-care device is currently available. In a preliminary study, ACR was found to gradually increase over time in dogs with acute kidney injury (AKI), similar to serum creatinine and SDMA levels.

Thus, the purpose of this study was to evaluate the clinical utility of urine ACR using a point-of-care device as an early detection urinary biomarker in dogs with CKD, to identify the correlation between ACR and other formerly known CKD biomarkers, and to determine the cut-off value of ACR for diagnosing CKD in dogs.

Materials and Methods

Study design

Serum and urine samples were obtained from healthy dogs and dogs with CKD at five local animal hospitals after obtaining the dog owner's consent to use the samples for research purposes; the animal hospitals that participated in this study were Nine Animal Medical Center (Hwaseong-si, Gyeonggi-do, Republic of Korea), Times Animal Medical Center (Suwon-si, Gyeonggi-do, Republic of Korea), Songjeong Animal Medical Center (Gwangju-si, Gyeonggi-do, Republic of Korea), Yongkang Animal Hospital (Seoul, Republic of Korea), and Cheonan Animal Medical Center (Cheonan-si, Chungcheongnam-do, Republic of Korea). The sampling period was from February 2021 to June 2021 for admission for health and medical examinations.

In total, 100 dogs were selected for this study. Of the 100 dogs, 50 were healthy and 50 were diagnosed with CKD. Dogs with CKD were classified into four stages according to the disease severity based on the criteria proposed in the International Renal Interest Society (IRIS) guide (14). The CKD IRIS staging system is based on the serum concentrations of creatinine and SDMA in this study, and CKD was staged based on the serum creatinine concentrations. There were six dogs with IRIS stage 1, twenty-two dogs with IRIS stage 2, thirteen dogs with IRIS stage 3, and nine dogs with IRIS stage 4.

The concentrations of BUN, creatinine, and SDMA were measured in serum samples, and USG, UPC, and ACR were measured in urine samples (Fig. 1). All protocols in this study were approved by the Institutional Animal Care and Use Committee (PTB-2020-IACUC-003-A) prior to the experiment.

Inclusion and exclusion criteria

Healthy dogs were included in this study; for this if physical examination did not reveal any abnormalities, complete blood count (CBC), serum biochemical profile, radiography, or ultrasound were performed to check for any clinical signs of CKD. Dogs with CKD were diagnosed by a veterinarian based on the CBC, serum biochemical profile, radiography, or ultrasound, with kidney images being identified as abnormal

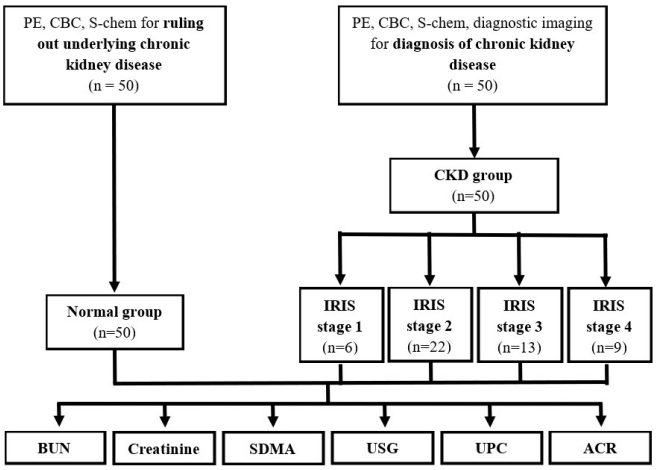

Fig. 1. Study design and population for the evaluation of ACR. PE, physical examination; CBC, complete blood count; S-chem, serum chemistry; CKD, chronic kidney disease; IRIS, international renal interest society; BUN, blood urea nitrogen; SDMA, symmetric dimethylarginine; USG, urine specific gravity; UPC, urine protein creatinine ratio; ACR, albumin creatinine ratio.

on the basis of IRIS criteria.

Dogs with hematuria, urolith, cystitis, or systemic underlying conditions associated with albuminuria such as occult neoplastic, infectious, immune-inflammatory, or non-renal cardiovascular diseases were excluded. But further, serum and urine samples left at room temperature for more than 24 h, insufficient amounts of samples, a condition that would have warranted a retest, and samples that were contaminated were also excluded.

Sample collection

Blood from the dogs was collected by venipuncture from the jugular vein at local animal hospitals. A serum separating tube (BD Vacutainer® SST™ Tube, Franklin Lakes, NJ, USA) was used to separate the serum. The tube was left to stand for 30 min and centrifuged at 3000 rpm for 15 min. Serum samples were stored at –70°C.

Urine samples were mostly collected via cystocentesis or few via catheterization at local animal hospitals. The collected urine was stored at 4°C within a week until use, and left at room temperature for 30 min before testing for USG, UPC, and ACR.

Retrieval of medical records

Clinical data including sex, breed, age, body weight, physical examination, and history of clinical signs were obtained from medical records.

The concentrations of BUN, creatinine, and SDMA were measured in the serum samples. Medical records for serum BUN, creatinine, and SDMA concentrations were obtained from local animal hospitals and critically reviewed to evaluate the minimum data base or results for renal function. In case of absence of medical records, the serum samples stored at –70°C were requested by IDEXX laboratories (IDEXX Laboratories Inc., Westbrook, ME, USA).

Urinalysis

For the collected urine samples, USG was quantitatively measured using a urine-specific gravity refractometer (ATAGO Inc, Tokyo, Japan). Zero adjustment was performed before measurement using distilled water.

The UPC was measured using a Catalyst One Chemistry Analyzer (IDEXX Inc.) according to the manufacturer's instructions. Calibration was performed before measurement. For UPC measurement, 300 μL of urine sample was put into the sample cup, the slides for protein and creatinine were loaded, and then, the same amount of diluent was added to the dilution cup. It took 11 min to obtain the results.

The ACR of the urine samples was measured using an A1Care®ACR analyzer (i-SENS Inc., Seoul, Korea) according to the manufacturer's instructions. Calibration was performed prior to the measurement. For ACR measurement, 40 μL of urine sample was injected into the cartridge through a pipette after pressing the collector to eject the analytical reagent into the cartridge. The cartridge containing the sample was then inserted into the device. The results were obtained within 7 min. The A1Care® ACR Cartridge measures albumin and creatinine concentrations by immunoturbidimetry and chemical colorimetry, respectively. In the first reaction unit, albumin in the urine sample and antibodies in the cartridge produce a complex. Turbidity increased according to the degree of production, and the albumin concentration was calculated based on the degree of turbidity. Urine creatinine level was calculated based on color alteration through the Jaffe reaction. ACR was calculated using the measured albumin and creatinine concentrations.

Statistical analysis

All data are expressed as mean and standard deviation. The Kolmogorov–Smirnov test was performed to assess normality. The Mann–Whitney U test was performed to evaluate the difference in renal biomarker values between healthy dogs and dogs with CKD. The Kruskal–Wallis test was performed to evaluate biomarker values according to IRIS stage. Spearman correlation analysis was performed to identify a correlation between biomarkers and ACR. Receiver operating characteristic (ROC) analysis was performed to evaluate clinical availability and the cut-off value. For statistical analysis,

SPSS ver. 26.0 software (IBM Co., Armonk, NY, USA) was used. Significance was defined as a p value < 0.05.

Results

Characteristics of the study population

The characteristics of healthy dogs and dogs with CKD in the study population are shown in Table 1. In healthy dogs as well as in dogs with CKD, no significant differences were observed between the sexes.

With respect to the breed distribution, Maltese (38%) was the most common breed in healthy dogs, followed by Poodle (16%), Shih-tzu (12%), Chihuahua (8%), Pomeranian (8%), Mixed (6%), Yorkshire terrier (4%), and other breeds (18%). In dogs with CKD, Shih-tzu (28%) was the most common breed, followed by Maltese (20%), Poodle (16%), Yorkshire Terrier (12%), mixed breed (10%), Chihuahua (4%), Pomeranian (2%), and other breeds (8%). There was no significant difference in breed distribution between healthy dogs and dogs with CKD.

The mean ages of healthy dogs and dogs with CKD were 9.67 ± 2.11 and 11.89 ± 2.99, respectively. The age of dogs with CKD was significantly higher than that of the healthy dogs ($p < 0.05$).

Body weights of healthy dogs and dogs with CKD were 5.22 ± 2.86 and 4.75 ± 1.97, respectively with no significant difference.

A comparison of renal biomarkers

The mean concentrations of serum BUN, creatinine, SDMA,

Table 1. Characteristics of the study population

Variables	Healthy dogs (n = 50)	Dogs with CKD (n = 50)	p-value
Sex, n (%)			0.544
Male	31 (62)	28 (56)	
Female	19 (38)	22 (44)	
Breed, n (%)			0.878
Maltese	14 (28)	10 (20)	
Poodle	8 (16)	8 (16)	
Shih-tzu	6 (12)	14 (28)	
Chihuahua	4 (8)	2 (4)	
Pomeranian	4 (8)	1 (2)	
Mixed	3 (6)	5 (10)	
Yorkshire terrier	2 (4)	6 (12)	
Others[a]	9 (18)	4 (8)	
Age (years, mean ± SD)	9.67 ± 2.11	11.89 ± 2.99	< 0.001*
BW (kg, mean ± SD)	5.22 ± 2.86	4.71 ± 1.97	0.717

CKD, chronic kidney disease.

[a]Others: Shiba, Miniature Pinscher, Beagle, Greyhound, Lhasa Apso, Jido dog, Pekingese, Cocker Spaniel.

*Indicates a significant difference between healthy dogs and dogs with CKD at $p < 0.05$.

Table 2. Comparison of renal biomarkers between healthy dogs and dogs with CKD

Variables	Healthy dogs		Dogs with CKD		Reference interval	p-value
	Mean ± SD	n	Mean ± SD	n		
BUN (mg/dL)	21.29 ± 5.66	50	64.06 ± 35.58	50	9-31	< 0.001*
Creatinine (mg/dL)	0.58 ± 0.25	50	3.13 ± 2.09	50	0.5-1.4	< 0.001*
SDMA (μg/dL)	11.07 ± 1.80	30	38.29 ± 15.94	28	0-18	< 0.001*
USG	1.048 ± 0.016	45	1.020 ± 0.038	48	> 1.030	< 0.001*
UPC	0.24 ± 0.27	50	3.77 ± 5.91	50	< 0.2	< 0.001*
ACR (mg/g)	22.48 ± 22.77	50	333.27 ± 66.96	50		< 0.001*

n = The number of analyzed samples of dogs.

CKD, chronic kidney disease; SD, standard deviation; BUN, blood urea nitrogen; SDMA, symmetric dimethylarginine; USG, urine specific gravity; UPC, urine protein creatinine ratio; ACR, albumin creatinine ratio.

*Indicates a significant difference between healthy dogs and dogs with CKD at $p < 0.05$.

and the mean values of USG, UPC, and ACR in healthy dogs and dogs with CKD are shown in Table 2.

In healthy dogs, the mean serum BUN concentration was 21.29 ± 5.66 mg/dL, whereas the dogs with CKD had 64.06 ± 35.58. Serum creatinine concentration was 0.58 ± 0.25 mg/dL in healthy dogs, and 3.13 ± 2.09 in the dogs with CKD. Serum SDMA concentrations in healthy dogs and dogs with CKD were 11.07 ± 1.80 and 38.29 ± 15.94 μg/dL, respectively. As for the urinary biomarkers, healthy dogs had a mean USG of 1.048 ± 0.016 and dogs with CKD had a USG of 1.020 ± 0.038. In healthy dogs, the mean UPC was 0.24 ± 0.27, and in dogs with CKD, UPC was 3.77 ± 5.91. ACR in healthy dogs was 22.48 ± 22.77 mg/g and in the dogs with CKD, ACR was 333.27 ± 266.96. There were significant differences in every renal biomarker between healthy dogs and dogs with CKD, including the ACR ($p < 0.05$).

Significant differences were observed in serum BUN concentrations between healthy dogs and dogs with CKD at every IRIS stage ($p < 0.05$). Moreover, dogs with IRIS stage 1 and stage 2 CKD had significant differences in serum BUN concentrations compared to dogs with IRIS stage 4 CKD ($p < 0.05$) (Fig. 2A). Serum creatinine concentrations were significantly different between healthy dogs and dogs with CKD at every IRIS stage ($p < 0.05$). In addition, there were significant differences in serum creatinine concentrations between every IRIS stage of CKD ($p < 0.05$) (Fig. 2B). There were significant differences in serum SDMA concentrations between healthy dogs and dogs with CKD at every IRIS stage ($p < 0.05$). In addition, dogs with IRIS stage 2 and stage 3 CKD had significant differences in serum SDMA concentrations compared to dogs with IRIS stage 4 CKD ($p < 0.05$) (Fig. 2C). In the case of USG, there were no significant differences among every IRIS stage of CKD, while significant differences were found between healthy dogs and dogs with CKD at every IRIS stage ($p < 0.05$) (Fig. 2D). In healthy dogs, UPC significantly differed between dogs with CKD in IRIS stages 2, 3, and 4 ($p < 0.05$), while there was no significant difference in stage 1. In addition, there were significant differences between dogs with IRIS stages 1 and 2 CKD and dogs with IRIS stage 4 CKD ($p < 0.05$) (Fig. 2E). In ACR, significant differences were observed between healthy dogs and dogs with CKD at every IRIS stage ($p < 0.05$). In addition, dogs with IRIS stages 1 and 2 CKD were significantly different from dogs with IRIS stage 4 CKD ($p < 0.05$) (Fig. 2F).

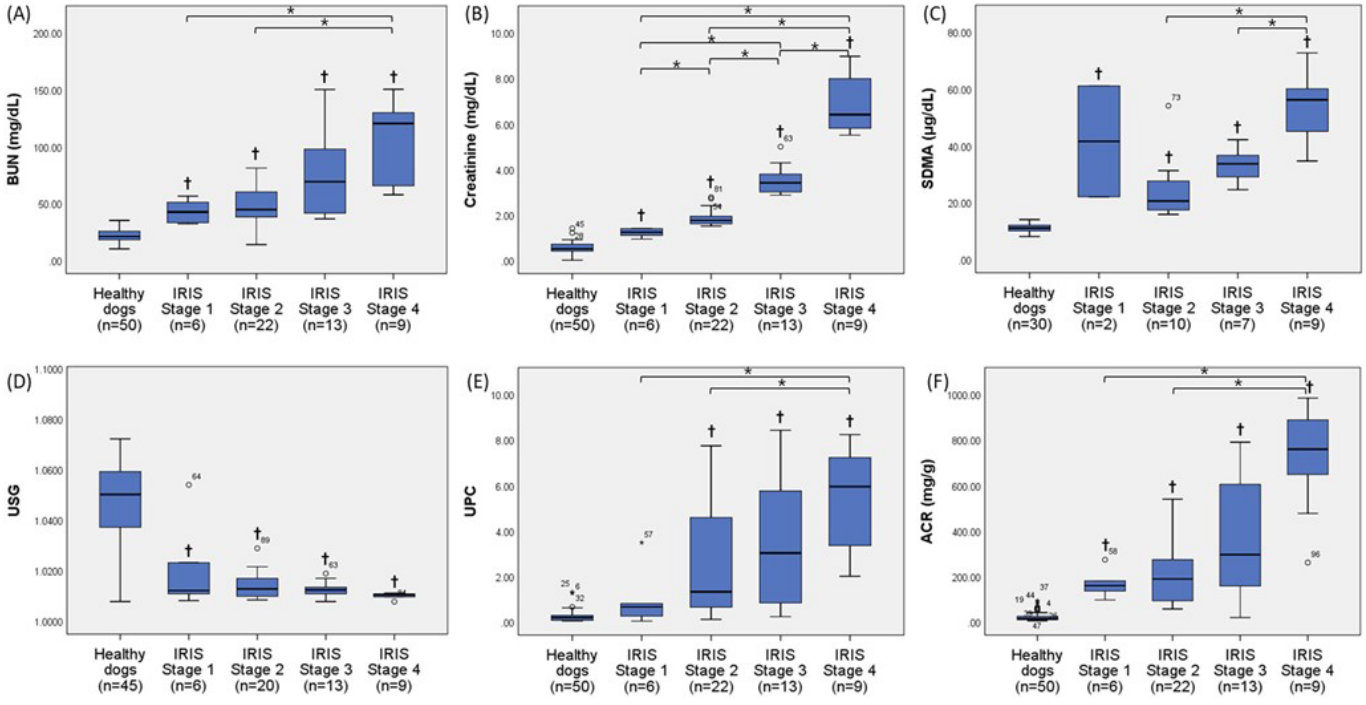

Fig. 2. Box plots of serum BUN (A), creatinine (B), SDMA (C) concentrations, USG (D), UPC (E), and ACR (F) in healthy dogs and dogs with CKD. †Indicates that there is significant difference from healthy dogs at $p < 0.05$, and *indicates that there is significant difference between two groups of dogs with CKD at $p < 0.05$. ACR, urine albumin/creatinine ratio; IRIS, international renal interest society; BUN, blood urea nitrogen; SDMA, symmetric dimethylarginine; USG, urine specific gravity; UPC, urine protein.

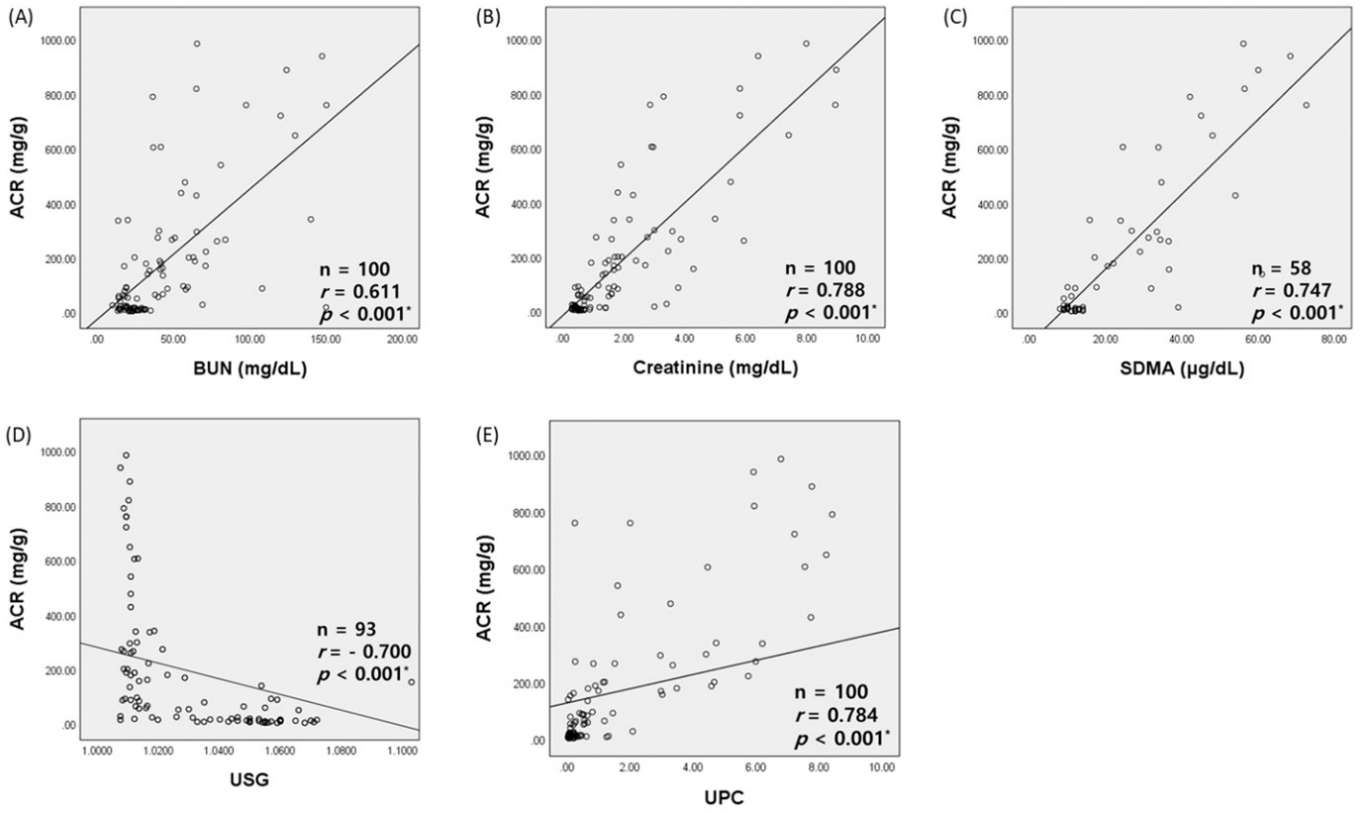

Fig. 3. Scatter plots with line regressions of the relationship between ACR and serum BUN (A), creatinine (B), SDMA (C) concentrations, USG (D), UPC (E). r: Spearman correlation coefficient, *p < 0.05. ACR, albumin creatinine ratio; BUN, blood urea nitrogen; SDMA, symmetric dimethylarginine; USG, urine specific gravity; UPC, urine protein creatinine ratio.

Comparison of ACR with other renal biomarkers

The correlation between ACR and other renal biomarkers is shown in Fig. 3. Moderate positive correlation was observed between ACR and serum BUN concentrations ($r = 0.611$, $p < 0.001$). ACR was strongly and positively correlated with serum creatinine concentrations ($r = 0.611$, $p < 0.001$), SDMA ($r = 0.747$, $p < 0.001$), and UPC ($r = 0.784$, $p < 0.001$). However, ACR was strongly and negatively correlated with USG ($r = –0.700$, $p < 0.001$).

The ROC curve analysis of ACR (Fig. 4) had an area under the curve (AUC) that was significantly different from 0.5 (AUC = 0.982, 95% CI: 0.963-1.000, $p < 0.001$). Fifty healthy dogs and fifty dogs with CKD were included in this analysis. The optimal cut off value of ACR was 64.20 mg/g with 94% sensitivity and 94% specificity.

The ROC curve analysis of ACR compared with other renal biomarkers is shown in Fig. 5. The ROC curve analysis of BUN, creatinine, SDMA, USG, UPC, and ACR had an AUC of 0.904 (95% CI: 0.810-1.000, $p < 0.001$), 1.000 (95% CI: 1.000-1.000, $p < 0.001$), 1.000 (95% CI: 1.000-1.000, $p < 0.001$), 0.052 (95% CI: 0.000-0.127, $p < 0.001$), 0.943 (95% CI: 0.869-1.000, $p < 0.001$), and 0.986 (95% CI: 0.963-1.000, $p < 0.001$) respectively. There were twenty-eight healthy dogs and twenty-eight dogs with CKD in this analysis.

Discussion

Generally, as CKD is irreversibly progressive, it is important to diagnose it early and provide proper medical application to slow the progression of the disease as described previously (5,11). Until recently, quantitative point-of-care devices for estimating ACR were not commercially available in veterinary medicine. Thus, in this study, the clinical diagnostic availability of ACR was evaluated through comparison with other previously used renal biomarkers.

In this study population, sex and breed distribution did not have an influence on the results obtained. In addition, previous studies have shown significant results regardless of sex and breed distribution when various breeds were included (1,8,17-19). In addition, dogs with CKD were significantly older compared with healthy dogs, and this result may be due to the

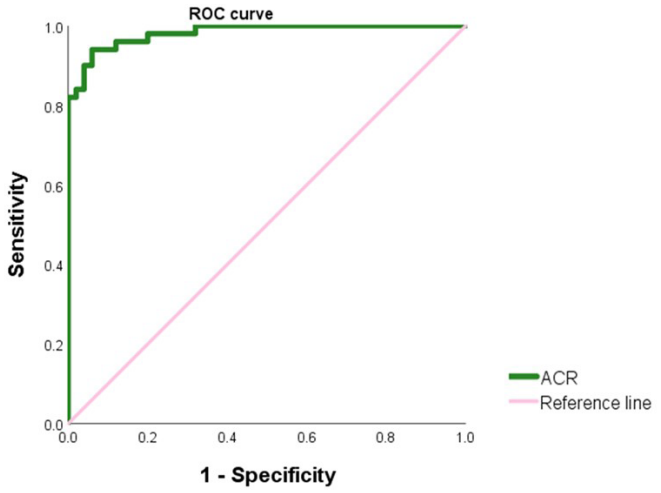

Fig. 4. ROC curve analysis for ACR. This analysis demonstrates sensitivity and specificity of ACR as diagnostic biomarker for CKD in 50 healthy dogs and 50 dogs with CKD. ACR had an AUC of 0.982 in this analysis (95% CI: 0.963-1.000, $p < 0.001$). ACR, albumin creatinine ratio; CKD, chronic kidney disease.

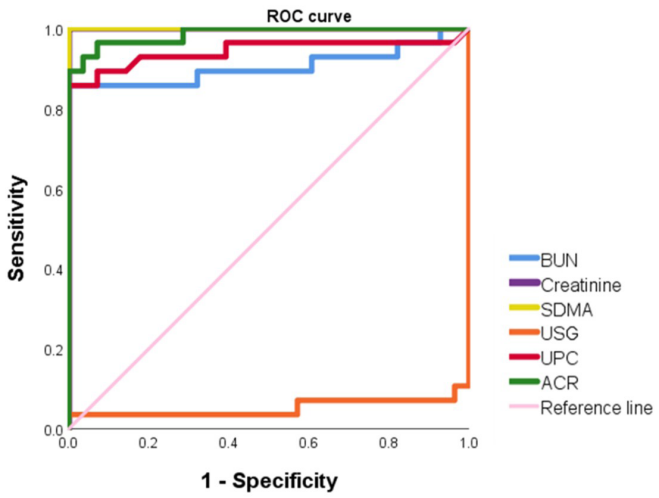

Fig. 5. ROC curve analysis of ACR compared with other renal biomarkers. This analysis demonstrates sensitivity and specificity of BUN, creatinine, SDMA, USG, UPC, and ACR as diagnostic biomarker for CKD in 28 healthy dogs and 28 dogs with CKD. The AUC of BUN, creatinine, SDMA, USG, UPC, and ACR were 0.904 ($p < 0.001$), 1.000 ($p < 0.001$), 1.000 ($p < 0.001$), 0.052 ($p < 0.001$), 0.943 ($p < 0.001$), and 0.986 ($p < 0.001$) respectively. ACR, albumin creatinine ratio; BUN, blood urea nitrogen; SDMA, symmetric dimethylarginine; USG, urine specific gravity; UPC, urine protein creatinine ratio; CKD, chronic kidney disease.

fact that CKD occurs mainly in older dogs (2). However, body weight did not significantly differ between healthy dogs and dogs with CKD, with lower body weights in dogs with CKD due to possible anorexia and chronic wasting conditions.

The ACR value of dogs with CKD was significantly different from that of healthy dogs; this result is similar to that obtained for other renal biomarkers in this study. Previous studies have demonstrated significant differences in ACR measured between dogs with CKD and healthy dogs using enzyme-linked immunosorbent assay (1,18). In addition, microalbuminuria could be confirmed by ACR through a semiquantitative assay in a previous study (19) and has been considered as glomerular dysfunction as urine albumin excretion is increased in patients with renal disease (12).

ACR showed significant differences between dogs with CKD IRIS stage I and healthy dogs, whereas there was no significant difference in UPC between dogs with CKD IRIS stage 1 and healthy dogs. This result indicated that microalbuminuria can be confirmed but not enough to be detected for total protein. Thus, the microalbuminuria assay is more sensitive and useful than the UPC test for detecting early-stage CKD in dogs (19).

However, ACR was not significantly different between every IRIS stage of CKD. ACR was numerically increased by IRIS stage in dogs with CKD. ACR is associated with the early detection of microalbuminuria, but not with staging. This can be related to the progressive reduction in the GFR (13). There was a significant strong positive correlation between ACR and advanced IRIS stage.

The significant correlation of ACR with other renal biomarkers suggests the potential clinical availability of ACR for diagnosing CKD in dogs. Previous studies have demonstrated a correlation between ACR and UPC (9,17). In this correlation analysis, serum BUN was only moderately and positively correlated with ACR, whereas other renal biomarkers were strongly significant. Serum BUN, which is a marker of CKD, decreases in cases of liver diseases and increases in case of high-protein meals or gastrointestinal bleeding.

ROC curve analysis of ACR in this study, patients can be diagnosed with CKD when ACR is greater than 64.20 mg/g with 94% sensitivity and 94% specificity. These results indicated that ACR is an excellent indicator of CKD. According to a previous report (4), the reference interval for the ACR was set as 0-19 mg/g in dogs. Among 124 healthy dogs, the median value was 3.0 mg/g with a total range of 0-48 mg/g (4), which is lower reference interval than the cut-off value of 64.20 mg/g in this paper. It might be due to differences in measurement methods for ACR and population. Therefore, further large-scale studies are necessary to evaluate the normal range of the ACR in dogs. In human medicine, microalbuminuria, which is defined as an ACR of 30-300 mg/g, is the earliest indicator of CKD (1,13). ROC curve analysis of the use of ACR for diagnosing CKD in dogs revealed that ACR was a useful

diagnostic biomarker for CKD at a similar level to other renal biomarkers except USG. There was a significance in the order of creatine, SDMA, ACR, UPC, and BUN in diagnosing CKD, whereas USG showed no significance in diagnosing CKD. This is because the CKD IRIS stage system is based on serum creatinine and SDMA concentrations, and USG can easily fluctuate according to the amount of drink or the time of collection.

In healthy dogs, those with USG of less than 1.030 had significantly higher ACR than those with USG of above 1.030, which might indicate CKD progression because decreased USG and elevated ACR are associated with microalbuminuria due to early glomerular dysfunction. For further investigation, follow-up observations are needed and should be verified in a larger number of patients.

However, microalbuminuria can be affected by physiological transient factors such as the posture of the patient, strenuous physical activity, hydration state, fever, stress, or pregnancy. In addition, urinary tract infections, inflammation, or severe hematuria, which can elicit false-positive results, should be ruled out prior to testing (12).

In the early stage of CKD, clinical signs are not common and are rarely noticeable (14), usually with normal serum creatinine and SDMA concentrations. In a preliminary study of AKI-induced dogs, there were dogs with elevated ACR, while serum creatinine and SDMA concentrations were both normal.

After transient increase, urinary tract infections, inflammation, or severe hematuria were excluded; when decreased USG with normal serum creatinine or SDMA concentrations; and diagnostic abnormal renal imaging with or without any clinical signs, the early stage of CKD diagnosis can be achieved by estimating the ACR.

Therefore, the early diagnosis of CKD can be accomplished by complementary diagnosis using other biomarkers including ACR.

However, there were limitations with respect to the study population. The number of healthy dogs or dogs with CKD was not perfectly matched with every biomarker as the serum and urine samples were not sufficient to measure serum SDMA concentrations or USG. The number of IRIS stages in each group was not sufficient to determine significance, especially for stage 1. Unfortunately, the number of dogs with IRIS stage 1 for SDMA was not sufficient to determine significance because of unnoticeable clinical signs in the early stage of CKD. Further investigations with an appropriate number of patients are required.

In conclusion, the measurement of ACR using a point-of-care device could be more useful than the UPC test because it is possible to diagnose CKD earlier with a small amount of urine in a short time. Therefore, ACR is considered a useful urinary biomarker for the early diagnosis of proteinuria in CKD. In addition, clear correlations were observed between ACR and other renal biomarkers. Based on this study, there was a diagnostic possibility of CKD when ACR was over 64.20 mg/g. Thus, the combined use of ACR and other renal biomarkers may be helpful for the early diagnosis and prevention of CKD in dogs.

Acknowledgements

The authors would like to give special thanks to Nine animal hospital, Times animal medical center, Songjeong animal medical center, Yongkang animal hospital and Cheonan animal medical center, and i-sens Korea Inc. for their assistance in this study.

This study result is a part of MS thesis for Hyun-Min Kang, Konkuk University.

Conflicts of Interest

The authors have no conflicting interests.

References

1. Bacic A, Kogika MM, Barbaro KC, Iuamoto CS, Simões DM, Santoro ML. Evaluation of albuminuria and its relationship with blood pressure in dogs with chronic kidney disease. Vet Clin Pathol 2010; 39: 203-209.
2. Bartges JW. Chronic kidney disease in dogs and cats. Vet Clin North Am Small Anim Pract 2012; 42: 669-692, vi.
3. Cobrin AR, Blois SL, Kruth SA, Abrams-Ogg AC, Dewey C. Biomarkers in the assessment of acute and chronic kidney diseases in the dog and cat. J Small Anim Pract 2013; 54: 647-655.
4. Falus FA, Vizi Z, Szabó KÉ, Müller L, Reiczigel J, Balogh N, et al. Establishment of a reference interval for urinary albumin-to-creatinine ratio in dogs. Vet Clin Pathol 2022; 51: 585-590.
5. Grauer GF. Early detection of renal damage and disease in dogs and cats. Vet Clin North Am Small Anim Pract 2005; 35: 581-596.
6. Harley L, Langston C. Proteinuria in dogs and cats. Can Vet J 2012; 53: 631-638.
7. Hebert LA, Spetie DN, Keane WF. The urgent call of albuminuria/proteinuria. Heeding its significance in early detection of kidney disease. Postgrad Med 2001; 110: 79-82, 87-88, 93-96. Erratum in: Postgrad Med 2002; 111: 23.
8. Jacob F, Polzin DJ, Osborne CA, Neaton JD, Kirk CA, Allen TA, et al. Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. J Am Vet Med Assoc 2005; 226: 393-400.
9. Kuwahar Y, Nishii N, Takasu M, Ohba Y, Maeda S, Kitagawa H.

Use of urine albumin/creatinine ratio for estimation of proteinuria in cats and dogs. J Vet Med Sci 2008; 70: 865-867.
10. Lees GE, Brown SA, Elliott J, Grauer GE, Vaden SL; American College of Veterinary Internal Medicine. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). J Vet Intern Med 2005; 19: 377-385.
11. Lees GE. Early diagnosis of renal disease and renal failure. Vet Clin North Am Small Anim Pract 2004; 34: 867-885, v.
12. Mogensen CE, Vestbo E, Poulsen PL, Christiansen C, Damsgaard EM, Eiskjaer H, et al. Microalbuminuria and potential confounders. A review and some observations on variability of urinary albumin excretion. Diabetes Care 1995; 18: 572-581.
13. Márquez DF, Ruiz-Hurtado G, Segura J, Ruilope L. Microalbuminuria and cardiorenal risk: old and new evidence in different populations. F1000Res 2019; 8: F1000 Faculty Rev-1659.
14. Perini-Perera S, Del-Ángel-Caraza J, Pérez-Sánchez AP, Quijano-Hernández IA, Recillas-Morales S. Evaluation of chronic kidney disease progression in dogs with therapeutic management of risk factors. Front Vet Sci 2021; 8: 621084.
15. Polzin DJ. Chronic kidney disease in small animals. Vet Clin North Am Small Anim Pract 2011; 41: 15-30.
16. Polzin DJ. Evidence-based step-wise approach to managing chronic kidney disease in dogs and cats. J Vet Emerg Crit Care (San Antonio) 2013; 23: 205-215.
17. Raila J, Brunnberg L, Schweigert FJ, Kohn B. Influence of kidney function on urinary excretion of albumin and retinol-binding protein in dogs with naturally occurring renal disease. Am J Vet Res 2010; 71: 1387-1394.
18. Smets PM, Meyer E, Maddens BE, Duchateau L, Daminet S. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. J Vet Intern Med 2010; 24: 65-72.
19. Whittemore JC, Gill VL, Jensen WA, Radecki SV, Lappin MR. Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs. J Am Vet Med Assoc 2006; 229: 958-963.