A DISSERTATION FOR THE DEGREE OF MASTER

Association of Renal Structural Parameters on Ultrasound with Estimated Glomerular Filtration Rate in Canine Chronic Kidney Disease

만성신장질환 개에서 초음파상 신장 구조적 지표 측정과 추정 사구체 여과율과의 비교

2018년 2월

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수의학과 임상수의학 (수의영상의학) 전공

이 시 헌

수의학석사학위논문

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Abstract

Association of Renal Structural Parameters on Ultrasound with Estimated Glomerular Filtration Rate in Canine Chronic Kidney Disease

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Many human studies have investigated the relationship between estimated glomerular filtration rate (eGFR) and renal structural parameters, including renal length (RL), renal cortical thickness (RCT), or renal cortical echogenicity (RCE), using ultrasonography (US). Currently, no studies have quantitatively evaluated canine renal structural parameters using US compared with eGFR for application in animals.

Because the distinction between the renal cortex and outer

medulla (OM) is clear in normal canine kidneys using US, the author hypothesized that the degree of differentiation between the cortex and OM would be related to renal function. It was also hypothesized that renal structural parameters estimated using US in canine chronic kidney disease (CKD) would have a linear relationship with eGFR; similar to results of human studies. The present study proposed methods for evaluating RCE using canine kidney characteristics via US (rather than through comparisons with other organs) and discussed the clinical significance of US parameters of the renal structure in canine CKD patients.

Symmetric dimethylarginine (SDMA) and serum creatinine concentrations were measured in all dogs; kidneys were evaluated using US within 1 month of the SDMA and creatinine concentration measurements. The US digital imaging and communications in medicine (DICOM) images were evaluated and RL, RCT, OM thickness (OMT), parenchymal thickness (PT; RCT + OMT), and RCE grade were measured. RCE was classified according to the degree of distinction of the renal cortex and OM. The measurements of RL, RCT, OMT, and PT were divided by body surface area for standardization (RLS, RCTS, OMTS, and PTS, respectively). All patients were classified into the normal kidney function (N) group or the CKD (C) group using the medical recording charts. Group C was subdivided according to whether the cortex and OM were distinct or not (C-1 and C-2, respectively). The differences in eGFR between group N and C-1 and C-2 and the tendency of the structural parameters (RLS, RCTS, OMTS, and PTS) in group C were analyzed based on eGFR using SDMA and creatinine

measurements.

A significant difference was observed in eGFR between groups N and C ($p < 0.0001$). The eGFR tended to decline from group N to C-1 and C-2 ($p < 0.0001$, St. J-T Statistic: −4.659). As the mean RCE grade (the mean RCE grades of the left and right kidneys) increased, the proportion of cases in group C tended to increase significantly ($p < 0.0001$). The mean RCE grade was negatively correlated with eGFR ($p < 0.001$). Positive correlations were also observed between RLS and eGFR and between PTS and eGFR in group C ($p < 0.05$ and < 0.02, respectively).

The RCE grade classified by the degree of distinction of the renal cortex and OM can be used as an objective method for evaluating RCE. Moreover, similar to human studies, renal structural parameters measured using US, including PTS and RLS, have a linear relationship with eGFR in canine CKD patients.

Keywords: dog; eGFR; renal cortical echogenicity; renal parenchymal thickness; cortex; outer medulla

Abbreviations

AUC	area under the ROC curve
BSA	body surface area
CART	classification and regression tree
CKD	chronic kidney disease
COM	RCT/OMT
eGFR	estimated glomerular filtration rate
OM	outer medulla
OMT	outer medulla thickness
OMTS	OMT/BSA
PT	parenchymal thickness (RCT + OMT)
PTS	PT/BSA
RCE	renal cortical echogenicity
RCT	renal cortical thickness
RCTS	RCT/BSA
RL	renal length
RLS	RL/BSA
ROC	receiver operating characteristic
SDMA	symmetric dimethylarginine
US	ultrasound, ultrasonography

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Introduction

Quantitative evaluation of the kidney includes measurements of the renal cortex or medulla thickness and volume according to renal function. These evaluations have been performed in many studies involving humans using various imaging modalities[1-8]. The results of these studies indicate that the thickness or volume of the renal cortex or medulla in chronic kidney disease (CKD) is linearly related to the estimated glomerular filtration rate (eGFR).

Quantitative assessment of the kidney is helpful in diagnosing or evaluating the prognosis of CKD patients in humans. However, to date, B-mode ultrasound (US) evaluation of canine kidneys has been limited to the examination of the renal margin, renal cortical echogenicity (RCE), and calculi and lesions (including cysts and neoplastic masses). Therefore, to determine whether the results of human studies are applicable to canine patients, the association of renal cortical thickness (RCT) and outer medulla thickness (OMT) to the International renal interest society (IRIS) stages in canine CKD patients was examined by the author in a previous study[9]. The results showed that RCT tended to decrease and OMT tended to increase according to the IRIS stage in canine CKD patients. One of the limitations of this study was that it was not compared with a clear standard of renal function such as GFR. Although various methods on calculating eGFR using objective patient information, including age, race, sex, weight, and hematological values, have been developed in humans[10−14]; such methods for determining eGFR in animals have not been developed. However, recently, it has been established that eGFR using symmetric dimethylarginine (SDMA) and creatinine concentration indirectly estimates GFR in animals[15]. The author conducted this study by using the eGFR more precisely than in the previous study. To author's knowledge, there are no detailed studies examining the relationship between renal structural parameters on US images (including, RCT and OMT) and GFR or eGFR in veterinary medicine.

This study presents a novel approach to the evaluation of RCE using US. The evaluation of RCE is typically performed by comparisons with adjacent organs (for example, the liver and spleen). However, this method is very subjective because it may differ according to the judgment of the US evaluator or as a result of abnormalities of the other organs being compared. In this study, the RCE grade was determined according to the distinction between the renal cortex and OM. Furthermore, based on this criterion, the author investigated the difference in RCE grade between patients with normal renal function and CKD and examined the relationship between RCE grade and eGFR.

The aim of this study is to investigate the relationship between eGFR and renal structural parameters using US, specifically in canine patients with CKD. In addition, criteria for objectively evaluating RCE using only the structural features of the canine kidney via US as opposed to comparisons with other organs are proposed. The validity of this grading was established using comparisons with eGFR.

Materials and Methods

1. Study design

Canine patients who visited the Veterinary Medical Teaching Hospital of Seoul National University (SNU VMTH) between June 2016 and August 2017 were selected for the study. Symmetric dimethylarginine and creatinine concentrations were measured and the patients were evaluated using abdominal US. SDMA concentration was measured by referring to the IDEXX Korea (IDEXX Reference Laboratories, Inc., Seongnam-si, Gyeonggi-do, Korea). Both SDMA and creatinine concentration measurement were performed on the same day. All patients were retrospectively classified into Normal (N) and CKD (C) groups based on the medical recording chart. Patients in group C were diagnosed with CKD in the Department of Internal Medicine of SNU VMTH, considering the patient's hematological values, persistence of clinical symptoms, urinalysis results and diagnostic imaging findings. Group C was retrospectively subdivided into C-1, if the renal cortex and the OM were distinguished using US, and C-2, if these structures were not distinguished using US. Cases with post-renal azotemia because of urinary tract obstruction, acute kidney injury, a dialysis history, a solitary kidney (for example, congenital or renal resection history), renal neoplasia, severe cortical structure deformation caused by multiple or large sized cystic lesions of the cortex, renal asymmetry (a difference in renal length (RL) greater than 5 mm between kidneys on the US findings); were excluded. Cases were also excluded if the SDMA and creatinine concentration measurements were not completed on the same day. Exclusion also occurred if the dates of the hematological measurements (SDMA and creatinine), and the US evaluation were more than 1 month apart.

2. Ultrasound evaluation of renal structural parameters

All US scans were obtained using commercial US products (Prosound alpha 7, Hitachi, Ltd., Tokyo, Japan), and either microconvex (4-10 MHz) or linear (4-13 MHz) probes were used. Measurements of RL, RCT, OMT, and PT were conducted on a DICOM image work station (INFINITT PACS, INFINITT Healthcare Co., Ltd., Seoul, Korea) with electronic calipers. The RL was measured as the maximal length in the dorsal plane from the cranial to the caudal pole. RCT and OMT were measured by considering that the cortex and the OM in US evaluations in normal dogs are easily differentiated[16]. Therefore, the RCT was measured at the uppermost hypoechoic section of the renal parenchyma, and the OMT was measured at the hyperechoic section immediately below the renal cortex in the sagittal plane. The PT was defined in this study as the sum of the RCT and OMT lengths. If the distinction between the renal cortex and the OM was difficult due to increased RCE in CKD, only PT (the length from the renal sinus to the capsule of the kidney) was measured. Examples of measuring RL, RCT, OMT and PT using US images are described in Figure 1. All of these parameters were divided by body surface area (BSA)[17] ($10.1 \times BW^{2/3} \times 10^{-4}$; BW is body weight in grams) for standardization, and denoted as RLS, RCTS, OMTS and PTS respectively. The parameters used for the analysis were the mean values measured in both kidneys.

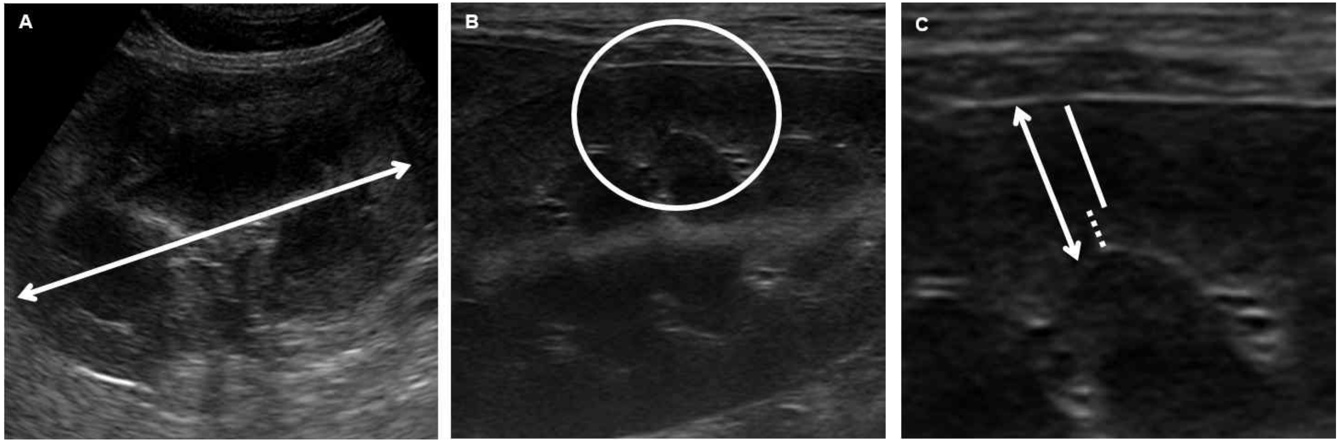

Figure 1. Measurement of renal length (RL), renal cortical thickness (RCT), outer medulla thickness (OMT), and parenchymal thickness (PT) on US images. RL is defined as the length of the long axis connecting the cranial and caudal pole of the kidney (double arrows), measured in the dorsal plane (A). (B) is the sagittal plane of the kidney on US and (C) is an enlarged image of the area indicated by the circle in (B). The solid line and the dotted line in (C) indicate the upper region of the renal sinus. The solid line is defined as the RCT and corresponds to the more hypoechoic part of this region. The dotted line is defined as the OMT and corresponds to the more hyperechoic part of this region. The double arrows define PT, which is the combined length of the solid line (RCT) and dotted line (OMT). For RCT, OMT, and PT, the vertical length from the renal sinus was measured.

3. Grading renal cortical echogenicity using ultrasound

RCE was also evaluated on DICOM images according to the distinction between the renal structures. It was divided into four grades according to the degree of differentiation of the renal cortex and OM. If the border between the renal cortex and OM can be clearly distinguished, it is classified as grade one. If the RCE is elevated, but the border between the renal cortex and OM is relatively clear, it is classified as grade two. If the border with the OM is ambiguous due to RCE elevation but it can be distinguished, it is classified as grade three. Finally, a grade four classification was given if it was difficult to distinguish the border of the OM due to severe RCE elevation. In this study, the RCE grade of each kidney was measured and the mean RCE grade was used in the statistical analyses. Examples for each RCE grade are shown in Figure 2.

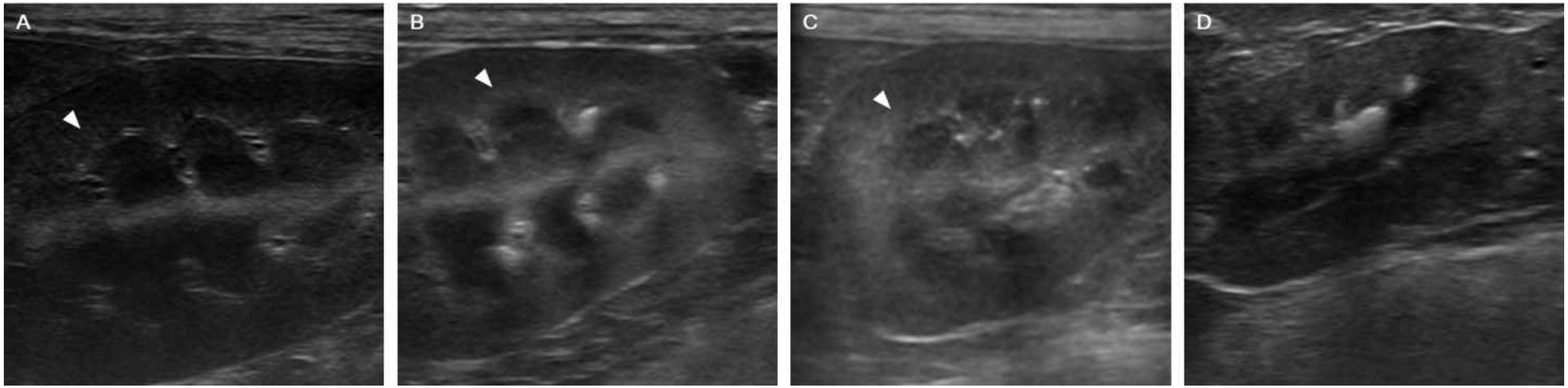

Figure 2. Renal cortical echogenicity (RCE) grade classification. Grade one is defined as a clear distinction of the border of the outer medulla (OM) and cortex without RCE elevation (A). Grade two, example (B), is defined as slight elevation of RCE, but the boundary with the OM is still distinct. In (C), if the RCE increases more than in (B); the boundary between the cortex and OM becomes ambiguous. Although unclear, if the boundary between the cortex and OM can be distinguished, as in this example, the authors classified the RCE grade as three. In (D), the RCE is elevated more than grade three, and the boundary between the cortex and OM cannot be distinguished. In this case, RCE grade was classified as grade four. If a patient's left and right kidney were classified as RCE grade one and two, respectively, the mean RCE grade (1.5) was recorded.

4. Quantification of renal function using eGFR

Although there are direct methods for measuring GFR, including renal clearance of inulin or exogenous creatinine and the use of radiolabeled markers; the clinical application of these methods is not easy[18]. In recent years, GFR has been examined using computed tomography and iohexol injections[19]; however, due to the cost and anesthesia protocol, this is also difficult to apply to clinical patients. Therefore, in this study, renal function was quantified using the eGFR, which was developed by Yerramilli et al.[15] based on SDMA and creatinine concentrations in animal patients. The method for eGFR presented in this patent is $(\text{creatinine})^{P} \times (\text{SDMA})^{Q}$. In this calculation, $P = -5$ to 0, and $Q = -2.5$ to 0 (excluding 0). The equation used in this study was $\text{eGFR} \approx (\text{Creatinine})^{-1.5} \times (\text{SDMA})^{-0.25}$, which was presented as the most ideal format[15].

5. Statistical analyses

All statistical analyses were performed using a commercial statistical software program (SPSS 23.0 for Windows; SPSS Inc., Chicago, IL, USA) and the R software for statistical computing (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/) using the package 'rpart' (Terry Therneau, Beth Atkinson and Brian Ripley (2017). rpart: Recursive Partitioning and Regression Trees. R package version 4.1-11.

https://CRAN.R−project.org/package=rpart) in R program. Differences in eGFR between the groups (N and C, N and C−1, N and C−2, C−1 and C−2) were compared by the Mann–Whitney test. The Jonckheere−Terpstra (J−T) tendency test was used to confirm the hypothesis that eGFR is reduced from the group N to C−1 and C−2. The Cochran–Armitage trend test was used to confirm whether the ratio of the group C tended to increase or decrease with increasing the mean RCE grade. The linear relationship between the mean RCE grade or renal structural parameters from the US images (including RLS, PTS, RCTS, OMTS, and COM) and eGFR were determined using linear regression. Classification and regression tree (CART)[20] analyses were used to optimally classify cases into group N or C. This process is used to investigate the factors that can predict normal or abnormal renal function. The following factors were input: eGFR, mean RCE grade, RLS and PTS. The most suitable factors for the analysis (including the inverse, square and square root) were assessed using the leave−one−out cross−validation[21]. CART was also used to classify cases into the N or C−1 groups. This classification was to determine whether normal and abnormal renal function can be predicted by the distinction between the renal cortex and the OM using US. The following factors were added to the classification and analysis of group N and C−1: eGFR, RLS, OMTS and COM; and these factors were also assessed using the leave−one−out cross−validation (including the inverse, square and square root factors). The area under the receiver operating characteristic (ROC) curves, (AUC), for each CART analysis was calculated. $p < 0.05$ was considered significant.

Results

1. Study population

During the study period, abdominal US, SDMA and creatinine concentrations were evaluated in 167 cases. Of these, 72 cases were excluded because the US evaluation and the hematological measurements were more than one month apart. In addition, 32 cases were excluded due to post renal azotemia (5), acute kidney injury (8), dialysis history (2), a solitary kidney (2), renal neoplasia (6), severe cortical structure deformations (5) and renal asymmetry (4).

Therefore, 63 cases satisfied the criteria for inclusion in this study. Thirty-five were male (five intact) and 28 were female (seven intact). Small sized breeds were the most common breeds participating in this study. The breeds included 23 Malteses, 8 Shih-Tzus, 7 Miniature poodles, 7 mixed breeds, 6 Yorkshire Terriers and 3 Pomeranians, 3 Schnauzers, 2 Cocker spaniels, 2 Miniature pinschers, 1 Chihuahua and 1 Welsh corgi. Of these, 27 were in group N and 36 were in group C. Group C was subdivided into the C-1 group (16/36) and the C-2 group (20/36) according to the degree of differentiation of the renal cortex and OM. Group N visited SNU VMTH for the following reasons: dermatologic disorders (1), cardiovascular system disorders (9), endocrine disorders (7), orthopedic disorders (1), neurological disorders (2), digestive system disorders (1), hepatobiliary and pancreatic disorders (3), hematological disorders such as anemia (3), respiratory system disorders (3), otitis externa (1), epistaxis (1) and one other reason which was not specified (1). Demographic data for these 63 cases are presented in Table 1.

2. Comparison of the group N and C with eGFR

First, the differences in eGFR between the groups in this study were examined. There was a significant difference in eGFR between group N and C ($p < 0.0001$). Also there were significant differences in eGFR between group N and C−1 ($p < 0.002$), and group N and C−2 ($p < 0.0001$). However, no significant difference was found between group C−1 and C−2 ($p = 0.214$). The eGFR showed a tendency to decline ($p < 0.0001$, St. J−T Statistic: −4.659) from group N to C−1 and C−2. The comparison between groups (N, C−1 and C−2) in eGFR and box plots are shown in Figure 3. Figure 4 is a diagram showing the result of the J−T test.

Table 1. Demographic data of the study population in the normal (N) group (n = 27) and the CKD (C) groups: C−1 (n = 16), C−2 (n = 20).

Variables		N (n = 27) Median (range)	C (n = 36)	
			C−1 (n = 16) Median (range)	C−2 (n = 20) Median (range)
Sex (n)	F	3	2	2
	FS	8	6	7
	M	1	−	4
	MC	15	8	7
Breeds (n)	Maltese	9	9	5
	Shih−Tzu	2	4	2
	Miniature poodle	4	−	3

	Mixed breed	6	1	–
	Yorkshire Terrier	1	2	3
	Others*	5	–	7
Age (years)		11.07 (1.0–16.0)	10.88 (4.0–15.00)	12.85 (8.00–17.00)
Body Weight (kg)		5.23 (1.05–13.73)	4.28 (2.18–10.14)	4.29 (1.93–8.30)
SDMA (μg/dL)		14.11 (9.00–27.00)	21.38 (13.00–50.00)	30.60 (17.00–57.00)
Creatinine (mg/dL)		0.93 (0.35–1.41)	1.53 (0.74–4.07)	1.90 (0.60–4.95)
eGFR $(\text{Creatinine})^{-1.5} \times (\text{SDMA})^{-0.25}$		0.73 (0.53–0.93)	0.36 (0.05–0.83)	0.28 (0.03–1.04)
BUN		25.03 (9.00–34.00)	53.24 (8.00–228.00)	71.23 (17.90–283.90)
RCE grade		2.19 (1.00–3.00)	2.59 (1.00–3.50)	3.93 (3.50–4.00)
RLS		256.36 (19.83–466.75)	271.55 (175.41–331.45)	270.51 (166.30–343.51)

RCTS	11.32 (6.61−20.17)	10.74 (7.68−14.49)	−
OMTS	4.84 (2.21−7.03)	5.42 (3.80−8.39)	−
PTS	16.16 (8.82−26.98)	16.17 (12.87−22.29)	17.29 (10.99−30.32)
COM	2.38 (1.45−3.07)	2.08 (1.29−3.06)	−

*Schnauzer, Pomeranian, Miniature pinscher, Cocker spaniel, Chihuahua, Welsh corgi

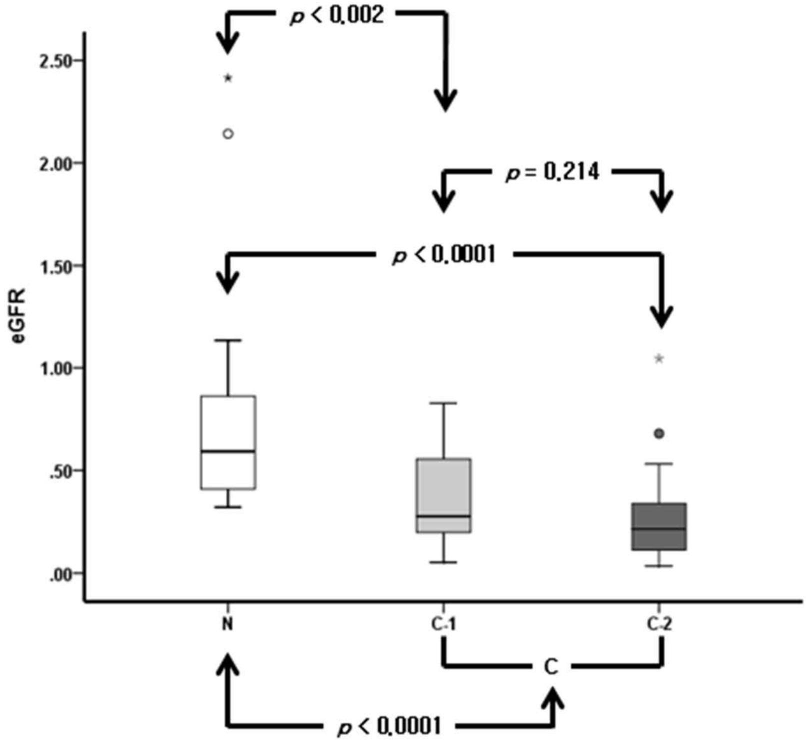

Figure 3. Box plots of eGFR in group N, C−1 and C−2. The central line in the box represents the median. The horizontal lines at the top and bottom of the box are the upper and lower quartiles. The whiskers on the top and bottom of the box represent the highest and the lowest data within the 1.5 interquartile range (IQR). The dots (○●) and stars (*) are considered outliers. The significance of the comparisons between the groups (*p* value) is indicated by the arrows.

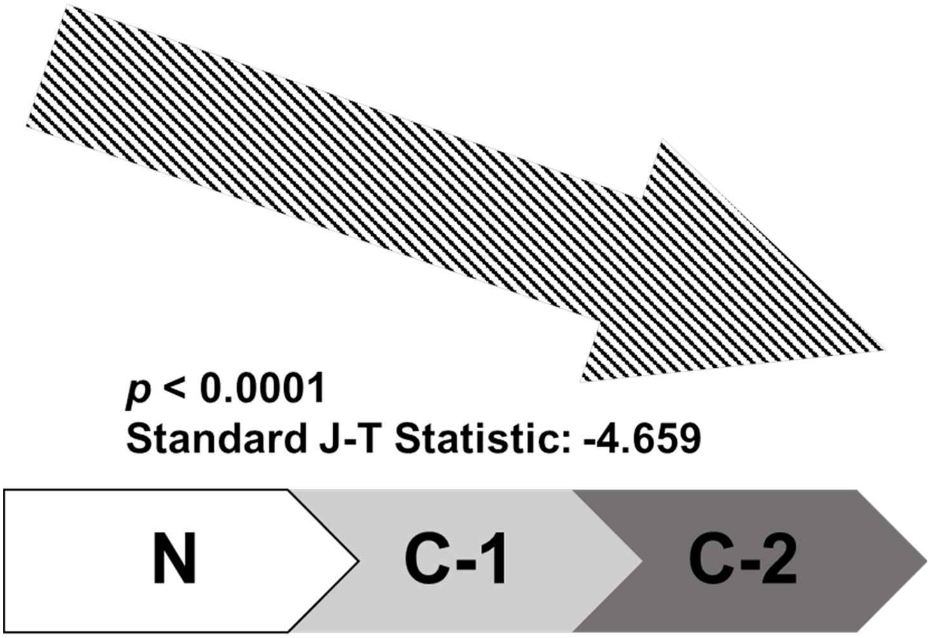

Figure 4. Result of the J−T trend test to confirm the tendency of eGFR according to group order: N, C−1, C−2. If the Standard J−T statistic is negative (St. J−T Statistic: −4.659), a decreasing tendency is indicated. The statistical significance was ($p < 0.0001$).

3. Comparison of mean RCE grade in group N and C

The mean RCE grade was divided into 7 stages ranging from 1 to 4 at 0.5 intervals (since it is expressed as the average value of the RCE grade of the left and right kidneys). Figure 5 shows the distribution of the mean RCE grade for each group. The ratio of cases in group C tended to increase with increasing mean RCE grade ($p < 0.0001$, Cochran–Armitage trend test). This suggests that the mean RCE grade increases with the deterioration of renal function. Table 2 summarizes the frequency of the mean RCE grade in groups N and C.

4. Correlation of mean RCE grade with eGFR

The mean RCE grade was negatively correlated with eGFR ($p < 0.0001$, $r = -0.474$). Figure 6 shows the scatter plots of eGFR and the mean RCE grade. The results of the simple linear regression analysis are also shown.

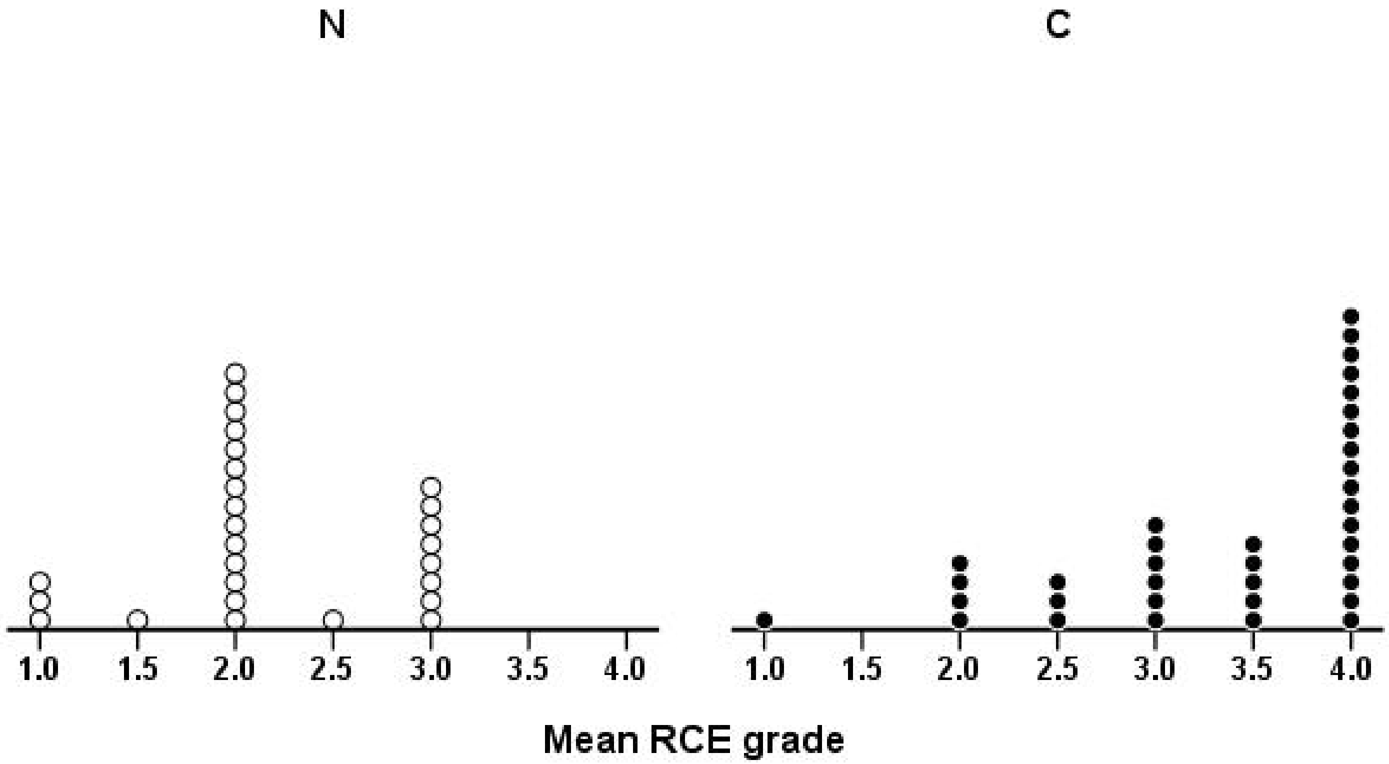

Figure 5. Dot plots of the mean RCE grade in the group N and C. Each dot (○ or ●) represents a case.

Table 2. Relationship between the frequency of Mean renal cortical echogenicity (RCE) grade of the group N and C

Group Mean RCE grade	N n (%)	C n (%)	Total n (%)
1	3 (11.1)	1 (2.8)	4 (6.3)
1.5	1 (3.7)	0 (0)	1 (1.6)
2	14 (51.9)	4 (11.1)	18 (28.6)
2.5	1 (3.7)	3 (8.3)	4 (6.4)
3	8 (29.6)	6 (16.7)	14 (22.2)
3.5	0 (0)	5 (13.9)	5 (7.9)
4	0 (0)	17 (47.2)	17 (27.0)
Total	27 (42.9)	36 (57.1)	63 (100)

The Cochran–Armitage trend test was used to examine the relationship between the ratio of cases in group C and the mean RCE grade ($p < 0.0001$).

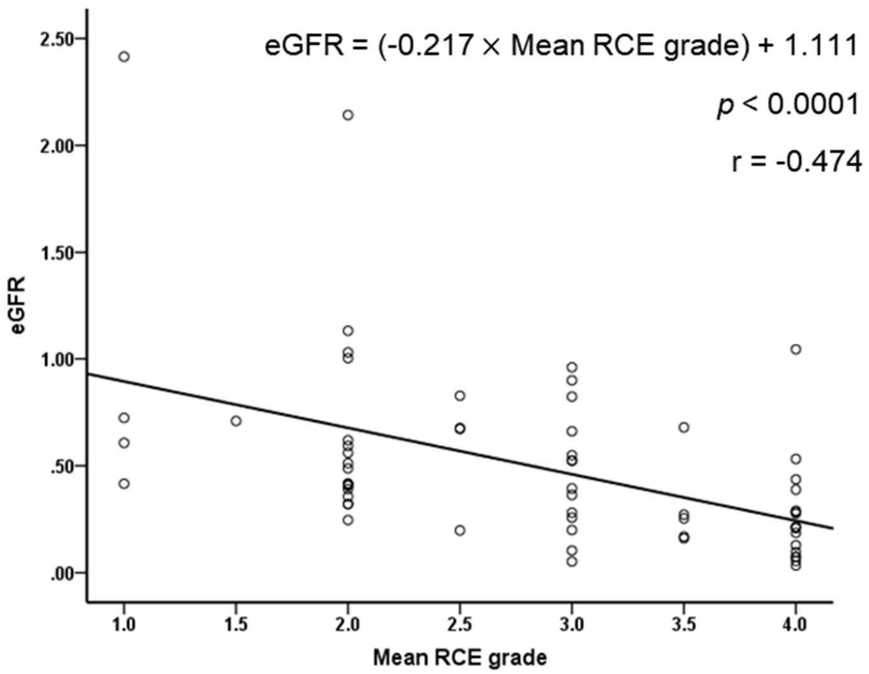

Figure 6. Scatter plot illustrating a significant linear correlation between eGFR and the mean RCE grade. Regression equation: eGFR = (− 0.217 × Mean RCE grade) + 1.111

5. Correlation of RLS and PTS with eGFR in group C; and RCTS, OMTS and COM with eGFR in group C1

In group C patients diagnosed with CKD (n=36), eGFR and RLS showed a significant positive linear relationship ($p < 0.05$, r = 0.329). There was also a significant positive linear relationship between PTS and eGFR in group C ($p < 0.02$, r = 0.415). However, there were no significant linear relationships between eGFR and RCTS, OMTS or COM in group C1 (n=16). Figure 7 shows scatter plots of eGFR, RLS and PTS and the results of the simple linear regression analysis.

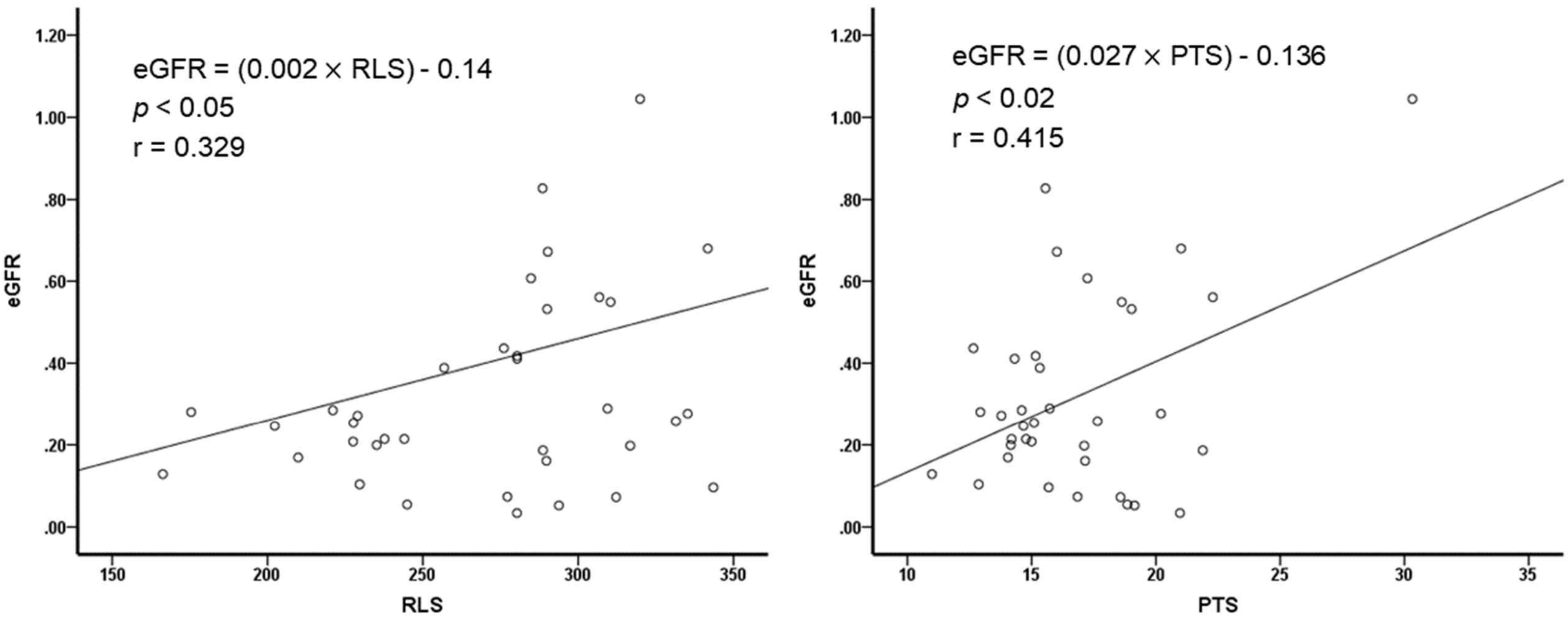

Figure 7. Scatter plots showing a positive linear relationship between eGFR and RLS or PTS in group C. The regression equations were: eGFR = (0.002 × RLS) − 0.14, and eGFR = (0.027 × PTS) − 0.136, respectively.

6. Classification and regression tree analysis of groups N and C

The CART analysis resulted in a tree diagram with five levels and 11 nodes, of which five of these were terminal nodes (Figure 8A). The CART analysis classified all of the cases in group N (n=25) and in group C (n=38). This was confirmed by the high area under the ROC curve, (AUC: 0.9943). The cut-off values for the cases in group N in this analysis were eGFR $\geq$ 0.305, mean RCE grade < 3.25, and RLS < 280.159. Alternatively, cutoff values were eGFR $\geq$ 0.305 and mean RCE grade < 3.25, and if the RLS cut-off value was $\geq$ 280.159, the cut-off values of $\geq$ 0.613 for eGFR and $\geq$ 16.584 for PTS were found. The ROC curve for the analysis is shown in Figure 9(A).

7. Classification and regression tree analysis of groups N and C1

The CART analysis was also used to classify the N and C−1 groups. The purpose of this analysis was to determine the factor most predictive of normal renal function (group N) and CKD when there was clear differentiation of the renal cortex and OM (group C−1). The results are shown in Figure 8(B). The CART analysis classified all cases in group N (25) and group C−1 (18), and this was confirmed by the high area under the ROC curve (AUC: 0.9931). Cut−off values for group N in this analysis were eGFR ≥ 0.301 and RLS < 280.159; alternatively, for the same eGFR cut−off (≥ 0.301), if the RLS cut off was ≥ 280.159, then cut−off values of ≥ 4.617 for OMTS and ≥ 2.205 for COM were found. The ROC curve for the analysis is shown in Figure 9(B).

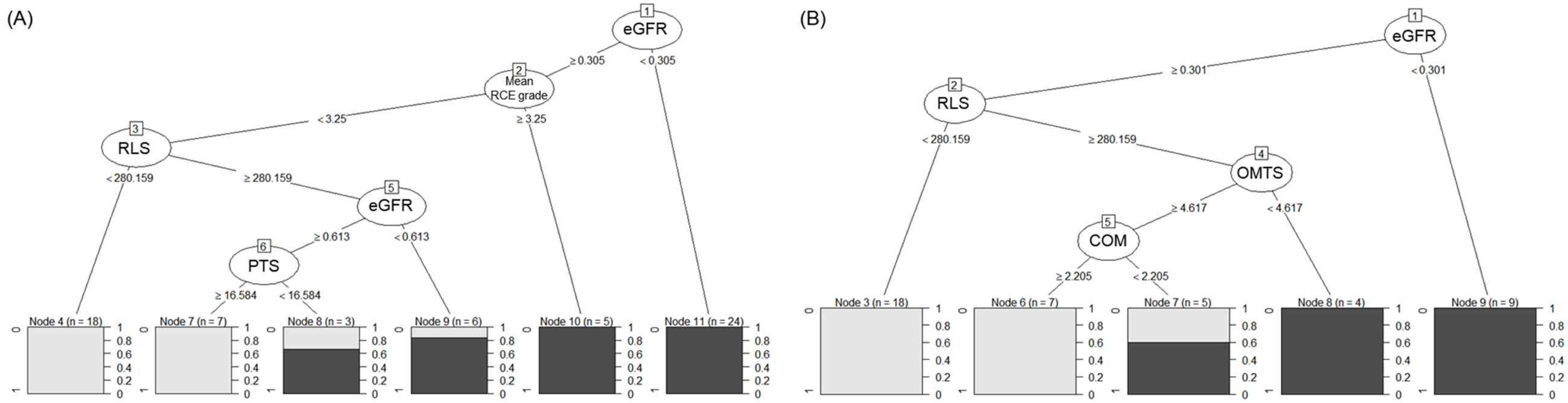

Figure 8. CART analysis for cases in in groups N and C (A) and in groups N and C−1 (B). The ellipses represent the splitting nodes and the rectangles represent the terminal nodes. The stacked bar plot at each terminal node represents the ratio of cases that were in the N group (light shading) and in the C or C−1 groups (dark shading).

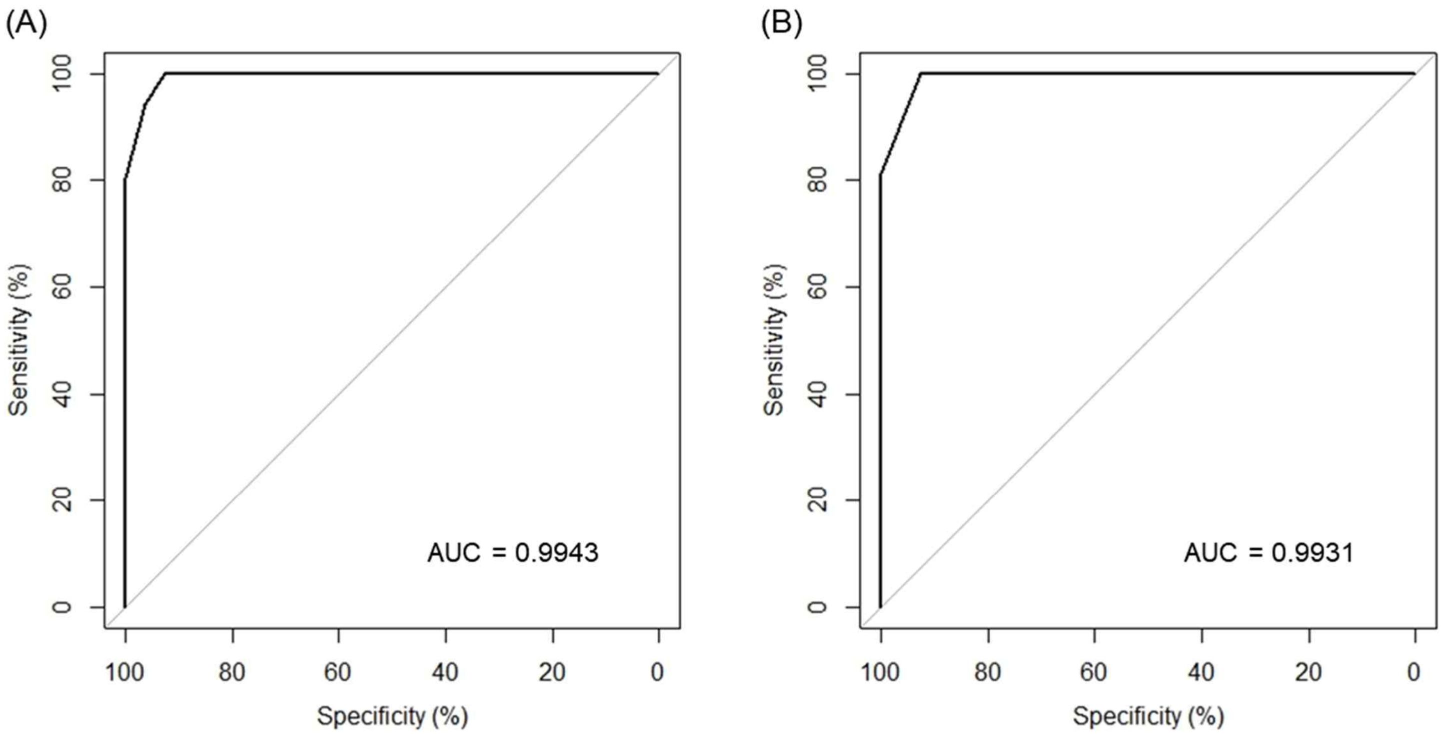

Figure 9. Receiving operating characteristic (ROC) curves for the CART analysis of groups N and C (A); and groups N and C−1 (B).

Discussion

In this study, there were significant differences in eGFR between groups N and C, N and C-1 and N and C-2; and the eGFR tended to decrease from group N to C-1 and C-2. The ratio of cases in group C tended to increase with increasing mean RCE grade; and the mean RCE grade showed a negative linear relationship with the eGFR. In group C, RLS and PTS showed a positive linear relationship with eGFR; and the relationship with PTS was more significant. Based on the cases and values from this study, the cut-off values that distinguished between group N and C or between group N and C-1 were obtained from the CART analysis.

In this study, the cases in group C were diagnosed with CKD based on the patient's hematological value, persistence of clinical symptoms, urinalysis results and diagnostic imaging findings; they were not based on the eGFR. Cases in Group C were categorized as either C-1 or C-2 according to whether the cortex and OM were able to be differentiated using US. Therefore, if the difference or tendency of eGFR between these groups is statistically significant, the grouping in this study can be considered valid. Significant differences in the eGFR were observed between groups N and C, groups N and C-1, and groups N and C-2; however, no significant differences were observed between groups C-1 and C-2. However, the J-T trend test showed a significant tendency for eGFR to decrease from group N to C−1 and C−2 groups. This supports the author's hypothesis that differentiation between the renal cortex and the OM using US would be difficult if renal function is decreased.

This hypothesis was also confirmed by the relationship between the mean RCE grade and eGFR. The elevation of RCE is associated with many other renal diseases and is nonspecific[22,23]. However, although RCE evaluation is one of the most important findings using US, no objective evaluation methods have been defined and interpretation reliant on subjective evaluation. One study attempted to objectively assess the degree of RCE elevation using comparisons to the liver in healthy dogs[24]. However, comparisons with adjacent organs are not objective RCE evaluation methods, especially in clinical patients. In this study, patients in group C were older than in group N and liver echogenicity was also elevated. The criterion used to evaluate RCE in this study was the differentiation between the renal cortex and the OM. The OM is a normal structure that is identifiable in the kidneys of dogs with normal renal function using US. It is known to be particularly recognizable in small breed dogs[16]. The sample used in this study consisted of mostly small breed dogs. In the samples of group N, the differentiation between the OM and the renal cortex was not difficult to find. Although classification of RCE (especially grades two or three), may involve a degree of evaluator subjectivity; the results indicated that the ratio of the number of cases in group C tended to significantly increase with increasing the mean RCE grade in this study. Also there was a significant negative linear relationship between the mean RCE grade and eGFR. Therefore, the RCE grading method presented in this study may be used to objectively evaluate RCE.

In this study, renal structural parameters on US images were standardized according to BSA and used in the statistical analyses. In humans, several studies have expressed renal structural parameters relative to patient body mass index, height, or weight[8,25]. When comparing the absolute values of the measured parameters with the standardized parameters, it was concluded that the standardized parameters have a greater association with the eGFR[25]. In veterinary medicine, the authors thought that standardization using BSA would be more reasonable than the height or weight of patients because of the large differences in specific breeds or individual patients in veterinary medicine.

It was statistically confirmed that RLS (RL standardized by BSA) had a linear relationship with eGFR. However, the relationship between eGFR and PTS was found to be more significant, implying that evaluation of the thickness of the renal cortex and OM, rather than the RL, better reflects renal function. These findings are comparable to the results of human studies. Hence the results suggest, that structural parameters specifically including the renal cortex are more closely related to the eGFR than the length of the kidney. Although trends were absent for more detailed structural parameters including RCTS, OMTS and COM in the C-1 group; due to a lack of samples, the author anticipates that the influence of these detailed parameters may be confirmed in the future. This is based on the existence of histological evidence including renal structural damage and fibrosis of the renal cortex due to the progression of CKD[26-29]. In particular, the COM of the kidney is a parameter that can be verified using US without the need for standardization or calibration. If a relationship between COM and eGFR was found, renal function could be deduced by measuring this ratio using US.

It is very important that the CART analysis showed a high AUC value when the study samples were classified. This suggests that the renal structural parameters can be used as classification criteria. If there were more cases, cut-off values representing normal kidney function using US could be accurately defined using the CART analysis.

There are some limitations of this study. Firstly, relatively few case studies were included. If there were more cases, the relevance of each structural parameter to the eGFR could be determined more precisely. The r values for the linear regression models including each parameter were not high. Secondly, in this retrospective study, the renal structural parameters or RCE grade, were evaluated on the US DICOM images. It is possible that evaluator bias influenced the measurement of the structural parameters or the classification of the RCE grade. Thirdly, renal function assessment was performed using eGFR rather than the measured GFR. The eGFR used in this study was based on the hematological value of SDMA and creatinine. Because these can be influenced by the hydration state of the patient on the measuring day, eGFR may not be able to fully represent the patient's renal function. Moreover, the study was based on only one eGFR formula that can be generally applied to small animals, such as dogs and cats. It is not a specific formula for use in dogs or cats. Various formulas for calculating eGFR have been established in humans, but not in veterinary medicine. It could be suggested that if different formulae for eGFR could be developed in the future that were specific to species or breeds for veterinary patients, the study could be conducted in a more diverse and precise way. Comparing of renal structural parameters using US could be used to determine the most appropriate eGFR representative of renal function. Lastly, the hematological values were not measured on the same day as the US evaluations. Therefore, the hematological values and the renal structural parameters derived using, may represent different renal functions. If the study was conducted using only cases where the US evaluation and the hematological measurements were conducted on the same day, more reasonable results of the relationship between renal function and renal structural parameters derived using US could be obtained. This is a limitation of retrospective research.

In conclusion, the degree of distinction between the renal cortex and the OM is closely related to renal function including eGFR; and the RCE grade defined in this study can be used as a method of objectively evaluating RCE. The author has also shown that renal structural parameters, including PTS and RLS, have a linear relationship with eGFR in canine CKD patients similar to studies in humans. Therefore, structural parameters of the kidney derived using US in canine CKD patients are good indicators of renal function; and PTS, including the thickness of the cortex and OM is more useful than RLS. Further studies using a larger number of cases could aid understanding of the relevance of more detailed renal structural parameters including RCTS, OMTS and COM to renal function (including eGFR). If then, US could be a diagnostic modality to predict the extent of renal dysfunction.

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국문초록

초음파상에서의 신장 길이나 피질의 두께, 피질의 에코와 같은 신장의 구조적 지표들과 추정 사구체 여과율과의 관계에 대한 많은 연구가 사람에서 보고된 바 있다. 지금까지는 개의 초음파상 신장의 구조적인 지표들에 대한 양적인 평가와 동물에 적용가능하도록 개발된 추정 사구체 여과율과의 관계에 대한 연구는 없었다.

우리는 정상 개의 초음파상 신장은 피질과 겉수질의 구분이 명확하다는 특징을 기반으로 피질과 겉수질의 구분 정도는 신장 기능과 관련이 있을 것이라고 가설하였다. 또한 사람에서의 연구와 마찬가지로 개의 만성신장질환 환자에서도 신장의 여러 구조적인 지표들이 추정 사구체 여과율과 선형의 관계에 있을 것이라고 가정하였다. 또한 이 연구를 통해서 만성신장질환 환자의 영상의학적 평가 시 피질 에코를 보다 객관적으로 평가하고 신장의 구조적 지표 평가에 대한 임상적 의의를 주장하고자 한다.

모든 개체에서 SDMA와 creatinine 농도를 측정하였으며 SDMA와 creatinine 농도를 측정한 날 전 후 한달 이내에 초음파 평가를 실시한 환자들만 연구에 포함시켰다. 초음파 DICOM image 상에서 신장 피질 두께 (RCT), 겉수질 두께 (OMT), RCT와 OMT의 합으로 정의하는 실질 두께 (PT)를 측정하고 피질과 겉수질의 구분 정도로 피질의 에코 상승 정도를 평가한 RCE grade를 평가하였다. RCT, OMT, PT는 BSA로 나누어 표준화 시켰으며 각각 RCTS, OMTS, PTS로 지칭한다. 모든 환자는 정상 그룹 (N)과 CKD 그룹 (C)으로 나누었으며 CKD 그룹은 다시 피질과 겉수질이 구분되는 정도에 따라 그룹 C-1, 그룹 C-2로 나누었다. 그룹 N과 그룹 C 간에 각 지표들에 대한 차이에 대해

알아보고 그룹 C에서 추정 사구체 여과율에 따른 지표들의 경향성을 알아보았다.

본 연구에서 구분한 그룹 N과 그룹 C에서는 추정 사구체 여과율의 유의미한 차이가 확인되었다. 추정 사구체 여과율은 그룹 N에서 그룹 C-1, 그룹 C-2로 갈수록 통계적으로 감소하는 경향을 보였으며, 좌측과 우측 RCE grade의 평균인 Mean RCE grade가 높아짐에 따라 그룹 C의 비율이 증가하는 경향이 확인되었고, 추정 사구체 여과율과의 음의 상관관계도 확인할 수 있었다. 또한 그룹 C-1과 그룹 C-2에서 RLS와 PTS는 추정 사구체 여과율에 따른 양의 상관관계를 확인할 수 있었다.

본 연구에서는 피질과 겉수질의 구분 정도로 단계를 나눈 RCE grade로 피질 에코 상승 정도를 평가하였으며 이는 피질 에코 평가의 객관적인 방법이 될 수 있다. 또한 개의 만성신장질환 환자에서 초음파 영상학적 평가 시 RLS나 PTS와 같은 구조적인 지표는 사람에서의 연구에서처럼 추정 사구체 여과율과 선형 관계에 있다.

주요어: 개, 추정 사구체 여과율, 신장 피질 에코, 신장 실질 두께, 피질, 겉수질