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Diagnostic assessment of two-dimensional shear wave elastography in relation to dimethyl arginine levels in dogs with chronic kidney disease

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ABSTRACT

Background: In veterinary medicine, previous studies regarding the diagnostic performance of shear wave elastography (SWE) in chronic kidney disease (CKD) are not consistent with each other. Moreover, there has been no study evaluating the relationship between symmetric dimethyl arginine (SDMA) concentration and renal shear wave velocity (SWV) using two-dimensional SWE (2D SWE) in dogs with CKD.
Objectives: This study aimed to evaluate the diagnostic capability of 2D SWE in dogs with CKD and to assess the relationship between renal SWV and SDMA concentration.
Methods: Dogs with healthy kidneys and dogs with CKD underwent 2D SWE and SDMA assay. Renal stiffness was estimated as renal SWV in m/s.
Results: SDMA concentration had a weak positive correlation with the left ($r = 0.338$, $p = 0.022$) and right renal SWV ($r = 0.337$, $p = 0.044$). Renal SWV was not significantly different between healthy kidney and CKD groups in the left ($p = 0.085$) and right ($p = 0.171$) kidneys.
Conclusions: 2D SWE may could not distinguish between dogs with healthy kidney and dogs with early stage of CKD, but it would be useful for assessing the serial change of renal function in dogs.

Keywords: Biopsy; canine disease; chronic renal diseases; stiffness; 2D

INTRODUCTION

Chronic kidney disease (CKD) is the most common renal disease in older dogs, affecting approximately 0.5%–1.5% of all dogs [1,2]. CKD is typically progressive and irreversible [1,3]. Therefore, early diagnosis of CKD is essential for initiating proper treatment to prevent severe, progressive renal damage [2,3]. Although histological examination is considered the gold standard to diagnose CKD [3,4]. Renal biopsy is not routinely performed in

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clinical practice as it is an invasive procedure, and therefore, carries the risk of potential complications [3].

Laboratory tests have been used to detect CKD and monitor the progression of renal damage in clinical practice [3]. In 2019, the international renal interest society (IRIS) presented the modified IRIS staging system of CKD, which is based on fasting blood symmetric dimethyl arginine (SDMA) concentration. SDMA is sensitive for evaluating glomerular filtration rate and is less impacted by lean body loss than serum creatinine concentration; therefore, SDMA is considered a kidney excretory function biomarker in dogs [5,6]. However, SDMA concentration cannot assess the individual renal injury and requires repetitive blood sampling for monitoring renal function [5]. In contrast, conventional ultrasonography is used to screen patients with CKD by assessing the morphologic changes in the individual kidney and it is safe to perform [3]. In the advanced stage, hyperechoic renal parenchyma with a decreased size, pyelectasia, abnormal cortico-medullary junction, and irregular contour are typically found [7,8]. However, it is not quantitative and subjective [7]. Given the limitations of previous diagnostic modalities, advanced diagnostic tests for diagnosing CKD are needed.

Shear wave elastography (SWE) can estimate tissue stiffness based on the shear wave propagation speed generated by acoustic radiation force impulses [9]. Two-dimensional SWE (2D SWE) provides the 2D color map showing the tissue stiffness over a large area and the shear wave velocity (SWV) can be measured in velocity (meters/seconds) from the specific area [9,10]. Because the SWV in tissue increases according to the increase of stiffness, 2D SWE can offer information of tissue stiffness which can predict the pathologic changes in tissues [10].

CKD is histologically characterized by interstitial fibrosis, glomerulosclerosis, and tubular atrophy, which contribute to stiffening of renal tissues and it has encouraged researchers of human medicine to explore the feasibility of elastography in diagnosing CKD [3,4,7,11]. Although there are a number of studies regarding the evaluation of renal stiffness using shear wave elastography in human medicine [7,11-13]. The feasibility of shear wave elastography in diagnosing CKD remains quite controversial in humans [11]. In veterinary medicine, few studies have been reported to assess the application of SWE in CKD [2,14,15]. Previous studies revealed that CKD groups had a higher renal SWV than control groups in dogs and cats [2,15]. In contrast, another feline study revealed that there was no significant difference in renal SWV between groups [14]. Considering the controversy regarding feasibility of SWE in CKD, subsequent studies are warranted in veterinary medicine. Moreover, there has been no study evaluating the relationship between SDMA concentration and renal SWV using 2D SWE in dogs with CKD.

The purpose of this study was to determine the feasibility of 2D SWE in dogs with CKD and to assess the relationship between renal SWV and SDMA concentration. We hypothesized that SDMA concentration would be positively correlated with renal SWV and that renal SWV would be significantly higher in dogs with CKD than in dogs with healthy kidneys.

MATERIALS AND METHODS

This study included dogs with healthy kidneys and dogs with CKD who had undergone 2D SWE of the left and right kidneys at the Doctor Dog Animal Medical Center between January 31 and May 30, 2021. Owner's consent was acquired before performing the examination.

The study protocol was approved by the Institutional Animal Care and Use Committee of Chonnam National University (CNU IACUC-YB-2021-56).

The inclusion criteria were as follows: (1) conventional ultrasonography, 2D SWE, and laboratory tests including SDMA concentration; (2) reliable 2D SWE results; (3) no other conditions that could affect kidney stiffness, such as a large amount of ascites, were present; (4) no space-occupying renal lesions, such as renal neoplasia and hydronephrosis, were present; and (5) no congenital kidney diseases, such as renal hypoplasia, were present.

The selected dogs were classified into healthy kidney or CKD groups according to SDMA or creatinine concentration and ultrasonography findings. The SDMA concentration was measured using an immunologic-based assay (IDEXX, USA) on the same day as 2D SWE was performed. The criteria for classification into the healthy kidney group were as follows: (1) SDMA concentration < 14 μg/dL or creatinine concentration < 1.4 mg/dL and (2) the kidney had a normal ultrasonographic presentation.

The criteria for classification into the CKD group were as follows: (1) SDMA concentration ≥ 14 μg/dL, or serum creatinine concentration ≥ 1.4 mg/dL for more than 3 months, based on the modified IRIS staging system; and (2) at least two ultrasound findings compatible with CKD [1,15,16]: decreased kidney size or asymmetry, markedly decreased renal corticomedullary differentiation, increased renal echogenicity, renal cysts, and irregular renal contour [3,4,17]. Then, these dogs with CKD were classified into IRIS stages according to SDMA concentration.

The ultrasound machine (EPIQ 5; Philips, USA) was used for 2D SWE examinations. The methods were determined according to previous report and manufacturer's guidelines [18]. The transducer was placed on the abdomen without applying excessive pressure. A longitudinal image of the kidney was obtained through the intercostal approach for the right kidney and the subcostal approach for the left kidney. 2D SWE using the installed software (ElastQ Imaging; Philips) was performed (**Fig. 1**). The area with a confidence value of less than the confidence threshold (50%) was displayed as color defects in the color-coded map, and if sufficient color-coded areas were not acquired owing to the presence of color defects, it was considered a technical failure [19,20] (**Fig. 2**).

On the color-coded map, a circular 3-mm diameter region of interest (ROI) was then placed at the middle third of the renal cortex according to a previous human study [21]. In each ROI,

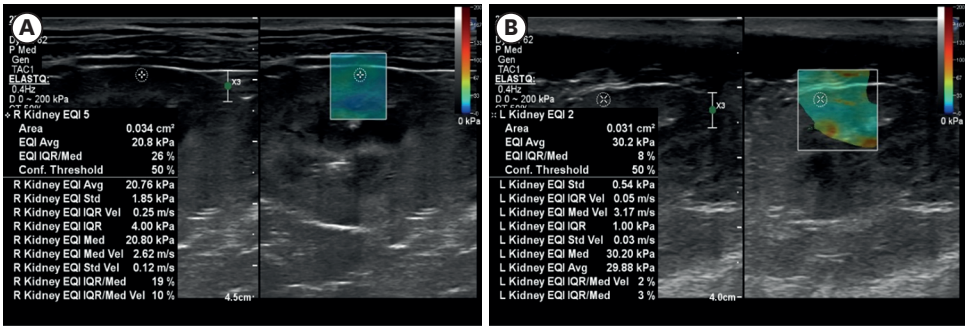

Fig. 1. Two-dimensional shear wave elastography of the healthy kidney (A) and chronic kidney disease (B). Renal shear wave velocity data are presented in the left corner of the display. In the color-coded map, a rectangular color box is placed over the longitudinal image of the kidney and a circular region of interest is located in the middle third cortical region.
Avg, average; Std, standard deviation; Vel, velocity; IQR, interquartile range; Med, median.

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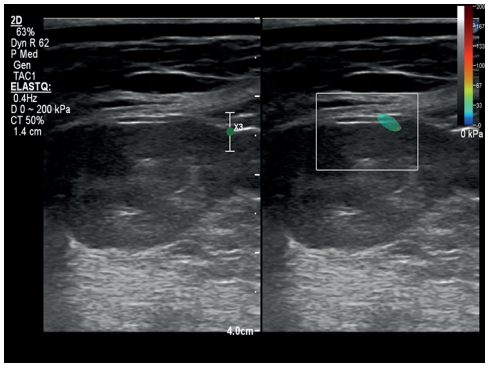

Fig. 2. Technical failure of the 2D shear wave elastography of the right kidney. The color-coded map (right) does not show enough color to locate an adequate region of interest. The color-coded image with a confidence value of less than the confidence threshold (50%) appears as color defects in this color-coded map.
2D, two-dimensional.

the interquartile range to median ratio (IQR/MED) was calculated automatically. When the IQR/MED was < 30%, the renal SWV in the ROI was accepted as valid data. In each dog, ROI placement and renal SWV measurements were performed until five valid data were obtained, and their median value was used as the representative renal SWV.

Statistical analyses were performed using a statistical program IBM SPSS Statistics 25 (IBM, USA) under the supervision of a statistician (J.K.K.). The Shapiro-Wilk test was used to assess whether the data showed a normal distribution. The correlation of renal SWV with the age and body weight of the dogs was analyzed using Pearson's correlation coefficient or Spearman's correlation coefficient. The correlation between renal SWV and SDMA concentration was analyzed using Spearman's correlation coefficient. Differences in renal SWV between healthy and CKD-affected kidneys and between the left and right kidneys were analyzed using the Student's *t*-test. The level of significance for all tests was set at $p < 0.05$.

RESULTS

In this study, 2D SWE was initially performed for 98 kidneys in 49 dogs; data obtained from 3 left kidneys and 13 right kidneys were excluded due to technical failures. Finally, the 2D SWE data of 46 left kidneys and 36 right kidneys obtained from 48 dogs were included: the healthy kidney group included 32 left and 28 right kidneys, and the CKD group included 14 left and 8 right kidneys, respectively. The IRIS stage of CKD was determined as stage 1 in 6 dogs, stage 2 in 7 dogs, and stage 3 in 1 dog. The mean age and SD of healthy kidney group and CKD group was 8.28 ± 3.88 years, and 13.58 ± 2.13 years, respectively. The breed, sex, and body weight of both groups are shown in **Table 1**. There was no correlation between renal SWV and age or body weight in either group (**Tables 2** and **3**).

Table 1. Breed, sex, and body weight of the healthy kidney group and CKD group

Clinical data	Healthy kidney group (n = 34)	CKD group (n = 14)
Breeds	Pomeranian (6), Poodle (6), Dachshund (5), Maltese (4), Mixed (3), Shih Tzu (3), Chihuahua (2), Yorkshire Terrier (2), Boston Terrier (1), Bull Terrier (1), Schnauzer (1)	Maltese (5), Yorkshire Terrier (3), Shih Tzu (2), Mixed (1), Poodle (1), Pomeranian (1), Schnauzer (1)
Sex	Neutered females (14), neutered males (14), intact females (5), intact male (1)	Neutered females (8), neutered males (6)
Body weight (kg)	5.35 ± 2.86	4.09 ± 2.02

Data are presented as mean ± SD or number.
CKD, chronic kidney disease.

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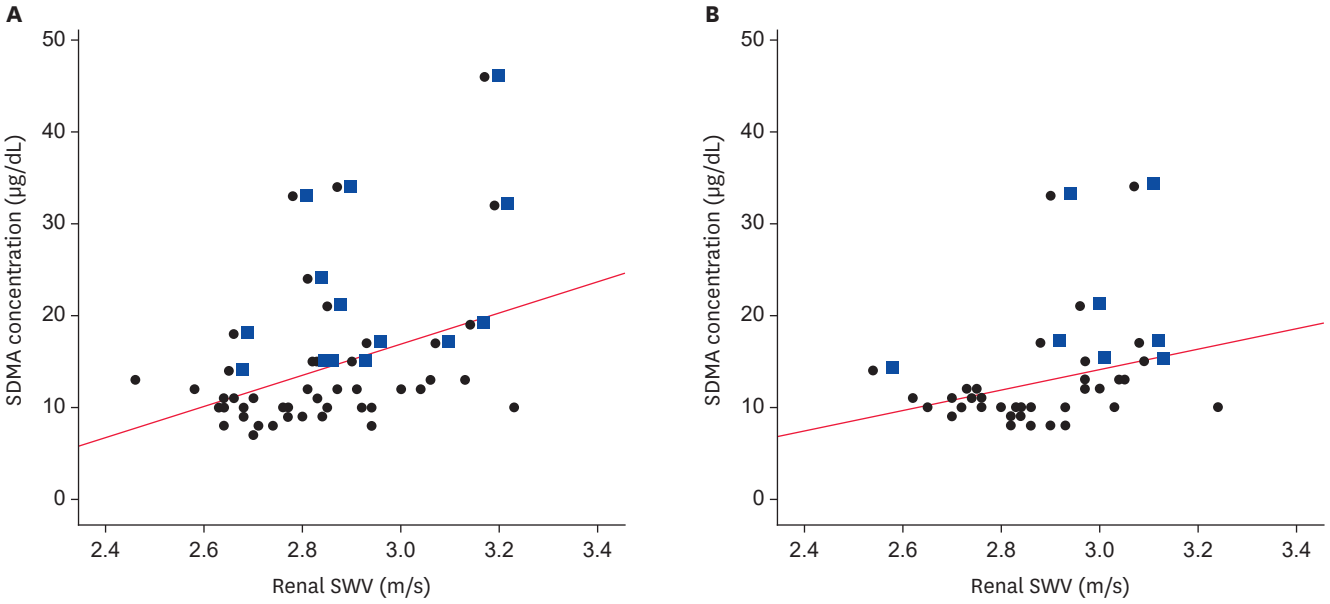

Fig. 3. Scatter plot of SDMA concentration and renal SWV of left kidney (A) and right kidney (B) in heathy kidney group and CKD group. SDMA concentration was moderately positive correlated with the left (r = 0.338, p = 0.022) and right renal SWV (r = 0.337, p = 0.044). Circle and square denote the healthy kidney group and CKD group, respectively. Solid line represents regression line.
SDMA, symmetric dimethyl arginine; SWV, shear wave velocity; CKD, chronic kidney disease.

Table 2. Correlation between age and renal shear wave velocity in the healthy kidney group and CKD group

Side of the kidney	Healthy kidney group (n = 34)		CKD group (n = 14)	
	Correlation coefficient	*p* value	Correlation coefficient	*p* value
Left kidney	0.249	0.169	0.020	0.945
Right kidney	0.230	0.238	0.214	0.610

CKD, chronic kidney disease.

Table 3. Correlation between body weight and renal shear wave velocity in the healthy kidney group and CKD group

Side of the kidney	Healthy kidney group (n = 34)		CKD group (n = 14)	
	Correlation coefficient	*p* value	Correlation coefficient	*p* value
Left kidney	0.202	0.268	−0.430	0.125
Right kidney	0.113	0.568	−0.429	0.289

CKD, chronic kidney disease.

The mean SDMA concentration and SD in the healthy kidney and CKD groups was 10.35 ± 1.59 μg/dL and 22.85 ± 9.69 μg/dL, respectively. SDMA concentration had a weak positive correlation with the left (r = 0.338, p = 0.022) and right renal SWV (r = 0.337, p = 0.044) (**Fig. 3**).

In the healthy kidney group, the mean renal SWV and SD was measured as 2.80 ± 0.17 m/s in the left kidney and 2.85 ± 0.14 m/s in the right kidney. In the CKD group, the mean renal SWV and SD was 2.90 ± 0.17 m/s in the left kidney and 2.93 ± 0.17 m/s in the right kidney. There was no significant difference between the left and right renal SWVs in both groups (healthy kidney group, p = 0.281; CKD group, p = 0.695). The renal SWV was not significantly different between the healthy kidney and CKD groups in the left (p = 0.085) and right (p = 0.171) kidneys.

DISCUSSION

To authors' knowledge, this is the first study revealed that renal SWV was positively correlated with SDMA concentration using 2D SWE. This study also found that there was no significant

difference of renal SWV between two groups, although the median value of renal SWV in CKD group was slightly higher.

In this study, renal SWV was significantly and positively correlated with SDMA concentration which increases with progression of CKD [3]. This result highlights that renal stiffness is associated with deterioration of renal function, similar to previous study of strain elastography [22]. With regards to positive relationship between renal SWV and SDMA concentration, 2D SWE could be useful for assessing the serial change in renal function.

Although the median value of renal SWV in CKD group was slightly higher. This study revealed that the renal SWV was not significantly different between the two groups. This result suggests that 2D SWE may could not distinguish between dogs with healthy kidney and dogs with early stage of CKD and it was not compatible with previous study [2]. This may be attributed to two possible reasons. First, in this study, renal SWV in the CKD group may have been underestimated because of the earlier stages of renal disease. In this study, the majority of the CKD group was classified as IRIS stage 1 (6/14, 42.8%) or 2 (7/14, 50.0%), unlike the previous canine study, in which CKD was classified as stage 2 (8/15, 53.3%) or stage 3 (7/15, 46.7%) [2].

Second, renal SWV might be affected not only by tissue fibrosis but also by changes in renal blood flow. In an experimental study using porcine kidney models revealed decreased renal stiffness after the ligation of the renal artery and suggested that the degree of blood perfusion influenced renal stiffness [23]. Moreover, this factor affecting renal SWV could have induced variable results in human studies regarding renal stiffness in CKD [11,24,25]. Similarly, we considered renal perfusion as a potential factor that could affect SWV. The canine kidney receives a rich blood supply relative to the organ weight of approximately 25% of the cardiac output [26]. Moreover, the renal cortex has a considerably higher blood flow than the renal medulla and there are dense peritubular capillary vessels in renal cortex that are connected to the glomeruli [27,28]. In dogs with CKD, blood flow in the peritubular capillary vessels decreases due to the sclerotic changes in the glomeruli [27]. Therefore, the decreased blood flow in the peritubular capillary vessels in the CKD group might have influenced the renal SWV in this study.

It was possible to perform the adequate 2D SWE examination and the calculation of the SWV in the left kidney in most dogs; however, technical failure occurred with 26.5% of right kidneys on the 2D SWE. In this study, the left kidney was scanned via the subcostal approach and the right kidney was scanned via the intercostal approach based on a previous study [18]. Because it was difficult to visualize 37.5% (3/8) of the right kidneys without compression using the subcostal approach in the study [18]. Moreover, interobserver agreement in 2D SWE of the right kidney was higher with the intercostal approach than with the subcostal approach [18]. In this study, the higher rate of technical failure with the right kidney could be related with the narrower intercostal space of small breed dogs in this study unlike the previous study used beagle dogs [18]. Similarly, in human medicine, children with a narrow intercostal space have a high failure rate when using the standard adult transducer [29]. Further studies about the effect of the intercostal scan approach of the kidney are needed in small breed dogs.

This study had several limitations. First, small number of dogs were included and most dogs with CKD were in IRIS stages 1 and 2; therefore, they did not represent the change in renal SWV in advanced stages. However, it helped to determine whether 2D SWE can be

used to diagnose early stage of CKD or not. Second, healthy, and CKD-affected kidneys were classified clinically without performing histologic examination. However, physical examination, blood test including SDMA and creatinine concentrations, and conventional ultrasonography were performed for assessing the kidney condition.

In conclusion, renal SWV had significant positive correlation with deterioration of renal function. However, 2D SWE may could not distinguish between dogs with healthy kidney and dogs with early stage of CKD. 2D SWE could be more useful for monitoring the renal function compared to the absolute criterion for the determination of CKD.

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