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RESEARCH ARTICLE

Usefulness of Contrast-enhanced Ultrasound in the Evaluation of Chronic Kidney Disease

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Abstract: ***Background*:** Contrast-enhanced ultrasound (CEUS) can provide more improved images of renal blood flow and much more information of both macro- and microcirculation of the kidney as compared to Doppler US.

***Objective*:** To investigate the usefulness of CEUS by analyzing differences in perfusion-related parameters among the three chronic kidney disease (CKD) subgroups and the control group.

***Methods*:** Thirty-eight patients with CKD and 21 controls who were age-matched (20−49 years) were included. Included CKD patients were stratified into three groups according to their eGFR: group I, eGFR ≥ 60 ml/min/1.73 m^2 (GFR category I and II); group II, 30 ml/min/1.73 m^2 ≤ eGFR < 60 ml/min/1.73 m^2 (GFR category III); and group III, eGFR < 30 ml/min/1.73 m^2 (GFR category IV and V). Comparisons with the controls (eGFR > 90 ml/min/1.73 m^2) were performed. Real-time and dynamic renal cortex imaging was performed using CEUS. Time-intensity curves and several bolus model quantitative perfusion parameters were created using the VueBox® quantification software. We compared the parameters among the CKD subgroups and between the CKD and control groups.

***Results*:** Eight patients were included in group I, 12 patients in group II, and 18 patients in group III. Significant differences were noted in the wash-in and wash-out rates between the CKD and control groups ($p = 0.027$ and $p = 0.018$, respectively), but not between those of the CKD subgroups. There were no significant differences of other perfusion parameters among the CKD subgroups and between the CKD and control groups.

***Conclusion*:** A few perfusion related CEUS parameters (WiR and WoR) can be used as markers of renal microvascular perfusion relating renal function. CEUS can effectively and quantitatively exhibit the renal microvascular perfusion in patients with CKD as well as normal control participants.

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1. INTRODUCTION

Chronic kidney disease (CKD) is defined as renal structural or functional abnormality for at least 3 months [1, 2]. The National Kidney Foundation recommends CKD staging based on the cause, GFR category, and albuminuria category [2]. In addition, the GFR categories of CKD have been defined based on the GFR calculations [2].

Functional and structural impairments are closely interrelated in CKD [3]. Therefore, both structural and functional information is required for the diagnosis of CKD. The information of structural change can be obtained from current imaging modalities, such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) [3]. Contrast-enhanced CT and dynamic enhanced MRI can provide functional information by measuring renal blood flow (RBF) and GFR, however, there is a limitation due to the potential hazard of contrast material to dysfunctional kidney [4]. Radionuclide studies also have a limitation due to radiation hazard and low resolution [3].

Functional MRI can be applied for the evaluation of CKD but it is limited in a clinical setting due to lack of standardized sequences, postprocessing software and models [5]. Doppler US without contrast agent can provide the information on the blood flow of large vessels but has a limitation in the evaluation of microvasculature. CEUS is cost-effective, non-toxic, and provides much more information on both macro- and microcirculation [6].

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CEUS can improve the imaging of RBF and lesional vascularity in real-time with exquisite temporal and spatial resolution [7-16]. Although CEUS can easily demonstrate the severity of renal cortical microvascular perfusion deficits [4, 14-21], there have been only a few studies to evaluate CKD using CEUS [17, 18, 21]. In our previous pilot study [21], we found no significant differences between the perfusion parameters among three groups of patients with CKD. We also found no correlation between the perfusion parameters and the estimated GFR (eGFR). Therefore, in this study, we included a normal control group and more patients with CKD, focusing on analyzing the perfusion parameters that affect CKD.

2. MATERIALS AND METHODS

The prospective study was approved by the Research and Ethics committee of our institution (No. C2015138), and written informed consent was acquired from each patient.

2.1. Patient Population

Thirty-eight consecutive patients with CKD and 21 healthy control were enrolled in this study over 1 year (between January 2018 and January 2019). All 59 patients were examined using conventional US and CEUS. The CKD group was diagnosed based on the histological or nephrological findings and was classified based on the GFR category [2]. Included CKD patients were stratified into three groups: group I, eGFR $\geq$ 60 ml/min/1.73 m^2 (GFR category I and II); group II, 30 ml/min/1.73 m^2 $\leq$ eGFR < 60 ml/min/1.73 m^2 (GFR category III); and group III, eGFR < 30 ml/min/1.73 m^2 (GFR category IV and V) [21]. The inclusion criterion for the normal control group was an eGFR > 90 ml/min/1.73 m^2. Both the CKD and control groups were matched for patient age (20–49 years) to eliminate its effects of age.

2.2. Conventional US Examination

A radiologist specialized in urogenital imaging for more than 16 years performed conventional grayscale and Doppler US of the kidney with an RS80A US machine (Samsung Medison, Seoul, Korea) using a 1–7 MHz probe.

2.3. CEUS Examination

US examination was performed in a more accessible kidney with a larger size and thicker cortex to reduce the investigation time [21]. SonoVue® (Bracco, Milano, Italy) was used as a US contrast agent. It was prepared for injection with an aseptic maneuver. Microbubbles suspension (2.4 mL) was administered intravenously using a dedicated syringe and, followed by an infusion of 10 mL 0.9% saline. When the contrast agent was injected, the images were collected simultaneously. For better image acquisition, the patients were instructed to take shallow breaths in supine or contralateral decubitus position.

CEUS was performed using the contrast-specific low mechanical index (MI = 0.08) mode. Image gain, focus point and scope were optimized before CEUS exam. During CEUS, we used screen split mode to display the grayscale and CEUS images simultaneously. Not only static images but also dynamic cine images were recorded for retrospective analysis. Satisfactory uptake usually lasted for 2 min in the kidneys [21].

2.4. CEUS Image Analysis

For the analysis of the renal perfusion images, we used a dedicated software package (VueBox®; Bracco Research, Geneva, Switzerland). The time-intensity curve (TIC) was obtained using the quality of fit [21].

Before calculation, we used automatic motion correction and the excluded out-of-plane images caused by patients' respiratory movements for more accurate analysis. (Fig. **1**) represents the process of offline analysis. For each sequence, one region of interest (ROI) was drawn. To minimize the influence of local perfusion heterogeneities, the ROI chosen corresponded to the largest area of the visible renal cortex on the surface of the kidney closest to the US probe [20, 21]. Intermittently visualized renal cortex from any reason was excluded from ROI. The microbubble concentration in each ROI was plotted against the time to yield a TIC, and the VueBox® software (Fig. **1**) provided the curve-fitting analysis. This curve was used to generate CEUS-derived parameters: peak enhancement (PE), time to peak (TTP), wash-in rate (WiR), wash-out rate (WoR), wash-in and wash-out area under the curve (WiWoAUC), rise time (RT), wash-in area under the curve (WiAUC), wash-out area under the curve (WoAUC), wash-in perfusion index (WiPI = WiAUC/RT), and fall time (FT).

2.5. Statistical Analysis

All analyses were performed using MedCalc 19.2.1 statistics software (MedCalc Software, Mariakerke, Belgium). The differences in perfusion-related parameters among the three CKD groups and the control group were evaluated with the one-way analysis of variance (ANOVA) test (CKD vs. control; four groups). A p-value of < 0.05 was considered statistically significant.

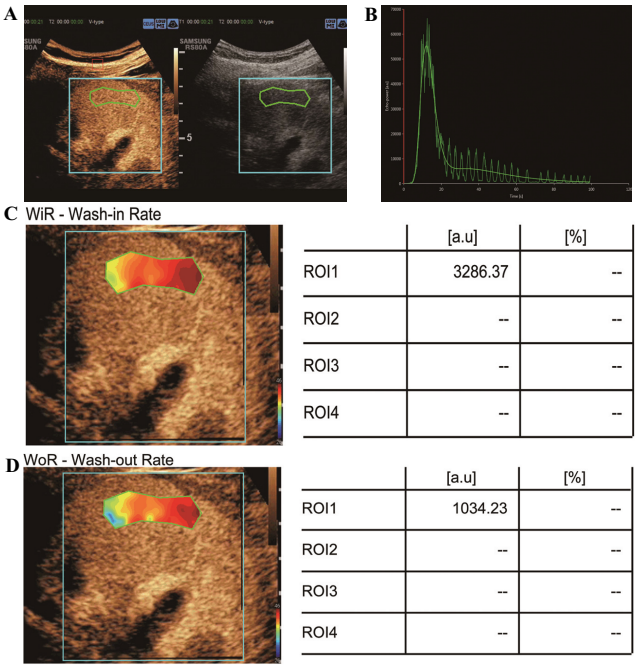
3. RESULTS

3.1. Patient Population

Data of 38 patients with CKD enrolled for the assessment of perfusion (13 women, 25 men; mean age, 37.2 ± 7.5 years; range, 21–49 years) were obtained. Group I included eight patients, group II included 12 patients, and group III included 18 patients. The control group included 21 adults (10 women, 11 men; mean age, 38.3 ± 9.5 years; range, 22–49 years).

3.2. Bolus Model Perfusion Parameters on CEUS

In all groups, the TIC of renal perfusion was an asymmetrical, single-peak curve with an ascending slope, a peak, and a descending slope. The ascending slope was steep, while the descending slope was flat (Fig. **2**) [17]. In the control



	[a.u]	[%]
ROI1	3286.37	--
ROI2	--	--
ROI3	--	--
ROI4	--	--

	[a.u]	[%]
ROI1	1034.23	--
ROI2	--	--
ROI3	--	--
ROI4	--	--

Fig. (1). Representative images of CEUS analysis. (**A**) A region of interest was drawn (green line) in the largest possible area of the renal cortex close to the US. (**B**) The software generated a time-intensity curve. This curve was used to generate CEUS-derived parameters. (**C**) and (**D**) obtained representative parameters are shown. **CEUS:** contrast-enhanced ultrasound. **US:** ultrasound. **WiR:** wash-in rate. **WoR:** wash-out rate. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

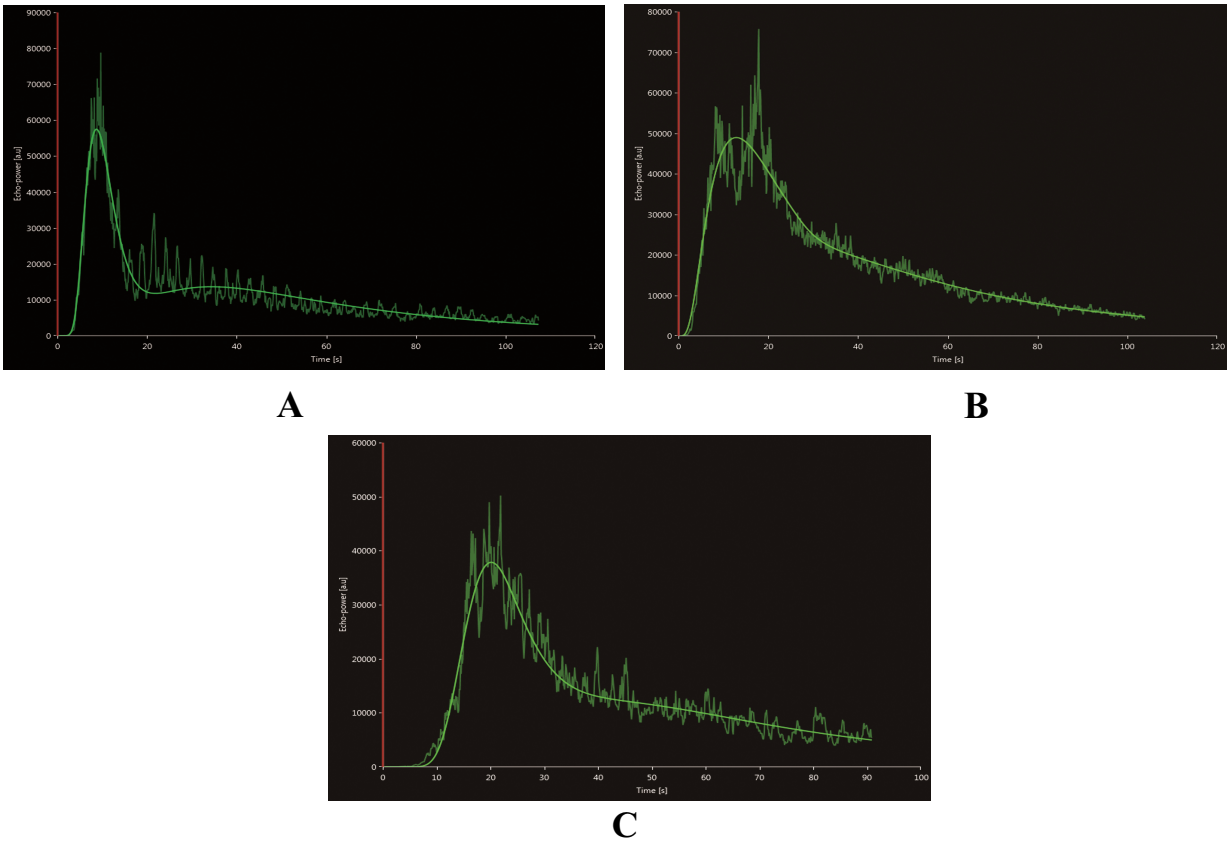


Fig. (2). Representative images of the time-intensity curve of renal perfusion. (**A**) perfusion curve in the control group. (**B**) perfusion curve in the early CKD group. (**C**) perfusion curve in the late CKD group. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

group, the ascending slope showed a trend of quick ascent before a descent after reaching the peak. In the TIC of the early CKD groups (group I, II), the ascending slope was flatter than that of the control group; it descended gradually after reaching the peak. In the late CKD group (group III), the ascending slope was much flatter, and the peak was lower than that of the other groups (Fig. **2**). On analysis of the perfusion-related parameters, no significant differences were observed among the four (three CKDs and one control) groups (Table **1**). No parameters significantly correlated with eGFR. However, there was a significant difference between the CKD and control groups (p = 0.027 in WiR and p = 0.018 in WoR) (Table **2**).

4. DISCUSSION

A method for evaluating renal microvascular perfusion that would be applicable in everyday clinic routine, even in the intensive care unit, would greatly improve the understanding of the pathophysiology of CKD as well as acute kidney injury [19]. The pathophysiology of CKD or acute kidney injury involves hemodynamic alterations, inflammation and renal tubular epithelial injury [22]. Epithelial injury leads to renal hypoxia with disturbances in nitric oxide pathways and eventually, microcirculatory dysfunction [23]. The reduction in renal microvascular perfusion, focal hypoxia, and inflammation leads to fibrogenesis and progression to CKD [14].

CEUS has significant advantages over other imaging modalities, such as MRI and CT, because it is portable, real-time, and cost-effective [6, 16]. Additionally, CEUS can be used for the evaluation of microvascular perfusion in patients with renal insufficiency, in whom contrast-enhanced MRI and CT are contraindicated [16].

None of the laboratory markers of CKD, such as serum creatinine, blood urea nitrogen, and urinary protein, are adequately sensitive [24, 25]. In addition, they do not satisfy the requirement of progression prediction and early detection of CKD. Because of this, renal biopsy is regarded as the best way to assess the pathologic changes including the severity of renal fibrosis. However, renal biopsy is inevitably invasive, susceptible to sampling errors, and impractical for longitudinal monitoring [24, 26]. Because of this limitation, a critical need has arisen for non-invasive and reproducible alternatives CEUS may be a valid candidate. Microvascular perfusion changes in CKD, decrease renal perfusion, and lead to enter fewer contrast microbubbles to the renal parenchyma. The microbubbles reflect perfusion in the microcirculation, and the renal microvasculature is a crucial contributor to the development of renal fibrosis and CKD progression. Therefore, the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines recommended the use of CEUS in patients with renal function impairment [27].

Both qualitative and quantitative approaches can be applied to the analysis of renal perfusion. Although a qualitative approach is easier (Fig. **2**), it is difficult to standardize. In contrast, the quantitative approach is complex, but it is good for standardization. Therefore, we applied a semi-quantitative approach. In this approach, various perfusion parameters were obtained to characterize the shape of the TIC. With this theoretical background, we aimed to investigate the usefulness of CEUS by evaluating the renal microvascular perfusion in CKD [21]. To the best of our knowledge, the studies evaluating CKD using CEUS, and their reports have been controversial [17-19, 21, 28]. Ma *et al.* reported that PI, TTP, and AUC may be used for diagnosing renal microvascular damage in patients with diabetes [27]. In our pilot study, there were no significant differences in the CEUS-driven perfusion parameters among the three groups of CKD [21]. In this study, we included a control group and compared it with the CKD group. Renal cortical microvascular perfusion was rapidly and clearly displayed on CEUS as the TIC in patients with CKD as well as the control participants. Moreover, there was a significant difference between the WiR and WoR in the control and CKD groups; the control group showed higher WiR and WoR than the CKD group.

The WiR is defined as the maximum slope between the time of onset of contrast inflow and the time of PE on the TIC [29]. The WoR is defined as the maximum slope between the TTP enhancement and the time of the end of contrast outflow on the TIC. Therefore, our results demonstrate that microvascular perfusion of CKD decreased as compared to the control group. Theoretically, not only WiR and WoR but also other perfusion parameters such as PI, TTP, and AUC should significantly differ between the CKD and control groups because each parameter is correlated with other parameters in the TIC. However, the other parameters were not significantly different in the CKD and normal control groups. This may have been due to a sampling error.

There are several limitations to our study. First, there was no correlation between histopathological results and perfusion parameters. Second, there was a limitation of generalization of our result using only a single type of contrast agent (SonoVue®), although there are no significant differences in mechanism between US contrast agents. Third, only a single ROI was used for the quantification of renal perfusion. If the volume of interest (VOI) was used, better assessment of kidney perfusion would be possible. However, the software (VueBox®) does not provide that feature. Lastly, other contributing factors of CKD, such as interstitial fibrosis was not evaluated in this study. Furthermore, we used a single US scanner and quantitatively analyzed the result using a commercial perfusion software (VueBox®). Therefore, further validation in future investigations using various US scanners and perfusion software programs as well as other functional properties, such as elastography, is warranted.

Nonetheless, we believe that the result of this study may demonstrate that CEUS can effectively and quantitatively exhibit decreased renal microvascular perfusion in patients with CKD as compared to normal controls. Furthermore, our study is more reliable because we matched the participant age (20–49 years) in both the CKD and control groups and excluded the age, which may influence the perfusion parameters.

Table 1. Parameters of renal microvascular perfusion according to the renal function.

Parameter	Control: *n = 21*	Group I: *n = 8*	Group II: *n = 12*	Group III: *n = 18*	*P*-value
PE	33882.12 ± 43623.24	15149.77 ± 15649.44	23772.97 ± 21861.98	21619.87 ± 20229.65	*P* = 0.432
TTP	15.80 ± 15.79	14.58 ± 6.99	14.92 ± 3.30	17.23 ± 8.68	*P* = 0.925
WiR	6955.40 ± 9711.89	2657.44 ± 2455.42	3655.25 ± 3491.58	3010.31 ± 2487.37	*P* = 0.175
WoR	3008.73 ± 4420.62	827.88 ± 646.49	1332.43 ± 1669.77	1177.89 ± 1108.80	*P* = 0.129
WiWoAUC	498235.01 ± 481622.43	308032.15 ± 406924.92	543437.42 ± 344852.37	474779.04 ± 477095.39	*P* = 0.694
RT	9.60 ± 6.88	10.68 ± 6.86	11.07 ± 3.91	11.03 ± 5.20	*P* = 0.859
WiAUC	159244.33 ± 171264.09	86873.82 ± 110794.13	159154.96 ± 119800.20	150999.75 ± 161568.13	*P* = 0.690
WoAUC	341371.67 ± 316561.13	221158.40 ± 296315.95	384282.49 ± 234325.36	323779.29 ± 318220.44	*P* = 0.687
WiPI	21455.80 ± 27335.00	9804.13 ± 10412.58	15371.33 ± 13641.42	13790.98 ± 12847.92	*P* = 0.443
FT	22.41 ± 17.36	29.15 ± 25.20	30.52 ± 20.39	24.77 ± 11.75	*P* = 0.586

Note: PE: peak enhancement
RT: rise time
TTP: time to peak
WiAUC: wash-in area under the curve
WiR: wash-in rate
WoAUC: wash-out area under the curve
WoR: wash-out rate
WiPI: wash-in perfusion index (WiAUC/RT)
WiWoAUC: wash-in and wash-out area under the curve
FT: fall time

Table 2. Parameters of renal microvascular perfusion in the control and CKD groups.

Parameter	Control: *n = 21*	CKD: *n = 38*	*P*-value
PE	33882.12 ± 43623.24	20937.67 ± 19660.39	*P* = 0.122
TTP	15.80 ± 15.79	15.94 ± 6.98	*P* = 0.961
WiR	6955.40 ± 9711.89	3139.69 ± 2784.30	*P* = 0.027*
WoR	3008.73 ± 4420.62	1153.01 ± 1227.37	*P* = 0.018*
WiWoAUC	498235.01 ± 481622.43	461356.02 ± 422635.47	*P* = 0.761
RT	9.60 ± 6.88	10.97 ± 5.09	*P* = 0.387
WiAUC	159244.33 ± 171264.09	140074.88 ± 139180.28	*P* = 0.643
WoAUC	341371.67 ± 316561.13	321281.17 ± 287963.41	*P* = 0.805
WiPI	21455.80 ± 27335.00	13450.70 ± 12482.24	*P* = 0.128
FT	22.41 ± 17.36	27.51 ± 17.73	*P* = 0.291

Data are presented as mean ± SD. * statistically significant
Note: CKD: chronic kidney disease
PE: peak enhancement
RT: rise time
TTP: time to peak
WiAUC: wash-in area under the curve
WiR: wash-in rate
WoAUC: wash-out area under the curve
WoR: wash-out rate
WiPI: wash-in perfusion index (WiAUC/RT)
WiWoAUC: wash-in and wash-out area under the curve
FT: fall time

In summary, there was a significant difference between the CKD and control groups in a few perfusion-related parameters (WiR and WoR). In other words, WiR and WoR can be used as markers of renal microvascular damage causing decreased perfusion in patients with CKD.

CONCLUSION

According to the data gathered from our study, it can be suggested that CEUS can effectively and quantitatively exhibit renal microvascular perfusion in patients with CKD as well as normal control participants. A few perfusion-related CEUS parameters (WiR and WoR) can be used as markers of renal microvascular perfusion related to renal function.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has been approved by the institutional review board (ethics committee of the "Chung-Ang University Hospital" Korea, IRB No. C2015138(1596).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All procedures followed were based on the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

CONSENT FOR PUBLICATION

Informed consent was obtained from all patients for being included in the study.

AVAILABILITY OF DATA AND MATERIALS

The source of data and materials in this study was Chung-Ang University Hospital. Data are available on request due to privacy or other restrictions

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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