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Value of ultrasound and contrast-enhanced ultrasound imaging in renal damage and treatment follow up of diabetic patients

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ABSTRACT

Background: it aimed to explore the value of ultrasound and contrast-enhanced ultrasound (CEUS) in evaluating renal function damage and renal artery blood flow changes in patients with diabetic nephropathy. Materials and ***Methods and Methods:*** 124 patients were rolled into an observation group receiving treatment with Shenkang injection (SKI) and a control group receiving conventional treatment. Various examination data from CEUS were compared between the two groups. ***Results:*** observation group showed notable improvements relative to control group in renal function indicators, 24-hour urinary albumin levels, plasma viscosity, erythrocyte sedimentation rate, and fibrinogen levels. CEUS further confirmed that observation group had better outcomes in terms of renal volume and blood flow parameters relative to control group. ***Conclusion:*** these findings suggested that SKI treatment can effectively enhance renal function and renal artery blood flow in patients with diabetic nephropathy, making it an effective clinical treatment option.

INTRODUCTION

Diabetes is a common chronic metabolic disease, and the patient number is increasing worldwide. Diabetic kidney disease (DKD), a complication of diabetes, is mainly caused by glomerulosclerosis caused by diabetic microangiopathy, which seriously threatens the survival time of patients [1,2]. According to statistics, about 4% of diabetic patients may develop DKD and diabetic neuropathy at the same time. Studies have found that changes in microcirculation hemodynamics are imperative factors leading to the occurrence of DKD, and genetic variants related to decreased glycolysis, mitochondrial dysfunction and DNA damage and continuous cell regeneration may be related to the prevention of diabetic microvascular complications [3-5]. There is a small amount of albumin present in the urine of patients with DKD in its early stages, which, if addressed promptly, can mitigate disease progression. However, patients who do not exhibit overt symptoms may overlook this critical early treatment window. As DKD advances to the clinical stage, patients typically manifest symptoms such as persistent proteinuria and hypertension, which are challenging to reverse. Without intervention during this phase, the condition gradually worsens, leading to the development of chronic renal failure. Ultimately, this progression may culminate in end-stage renal disease necessitating interventions such as dialysis or kidney transplantation [6-8]. Thus, timely treatment initiation and optimization of DKD management strategies hold paramount importance.

Shenkang injection (SKI) is a traditional Chinese medicine preparation, mainly containing salvia miltiorrhiza, astragalus, and safflower, which plays a role in protecting and repairing the kidney by regulating the physiological metabolic process in the body of patients, improving renal function, reducing tissue inflammatory reaction, kidney injury, reducing urinary protein excretion, and promoting the recovery of glomerular filtration rate [9,10]. Some studies have found that the release of inflammatory mediators in diabetic patients is increased, which can cause inflammatory response in kidney tissue, thus further aggravating renal lesions. During the development of DKD, abnormal proliferation of renal interstitial cells and renal tubular cells will occur, leading to increased fibrosis of renal tissue and weakened renal self-repair ability, which hinders the repair and regeneration of the kidney [11,12]. In SKI, however, some studies have shown that SKI can affect redox-related signaling pathways to reduce oxidative stress induced by advanced glycation end products (AGEs) and delay renal injury in DKD by regulating KELCH-like ECH-associated protein 1/transcription

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factor NF-E2-related factor 2/heme oxygenase-1 (Keap1/Nrf2/Ho-1) signaling pathway [13,14]. Some studies have also found that SKI can improve the clinical symptoms of patients. Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB), and plasminogen activator inhibitor -1 (PAI-1), D-dimer (D-D), 24-h urine total protein quantification (24h-UTP), urinary albumin excretion rates (UAER), and B2-microglobulin (β2-MG) levels were visibly reduced, delaying the progression of DKD [15,16]. In animal models, it has been found that SKI can inhibit inflammatory cytokines' release and increase antioxidant enzymes' activity in serum, and inhibit expressions of transforming growth factor-β1, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, collagen I, collagen III, endothelin-1 and laminin in the kidney of rats with functional failure. In the study of Qu *et al.* (2023) [17], it was found that Sprague Dawley (SD) rats had obvious symptoms of kidney injury after the model was established, and urinary albumin excretion (UAE), albumin/creatinine ratio (ACR) and blood urea nitrogen were visibly increased. After SKI treatment, it was found that the renal index, ACR and serum creatinine were visibly reduced, thereby improving the kidney injury and the verification reaction.

Ultrasound and CEUS technology is a non-invasive, convenient, and reproducible imaging examination methodology. Ultrasound technology uses the propagation and echo of high-frequency sound waves to produce images, and can detect the size and shape of the morphological structure of the kidney, the distribution of the cortex and medulla, etc. Ultrasound can also help detect abnormal conditions of the kidney such as masses, cysts, or stones [18,19]. CEUS adds contrast agent based on ultrasound examination, which can more clearly observe the blood flow of the kidney. In CEUS, the contrast agent is injected into the patient's body, and the contrast agent will enter the kidney with the blood flow and form a bright signal in the kidney, so that the renal artery blood flow and blood supply are more clearly visible [20,21]. Ultrasound and CEUS are imperative in the diagnosis and monitoring of DKD. Compared to other imaging modalities such as CT and MRI, ultrasound and CEUS techniques offer a safer and more cost-effective means to assess the morphological structure and functional status of the kidneys. Specifically, CEUS provides clearer images of renal artery blood flow and perfusion, thus used in the diagnosis and treatment monitoring of diabetic nephropathy [22,23]. Compared to other imaging techniques, the major advantage of ultrasound and CEUS lies in their non-invasive nature and avoidance of radiation exposure, making them an ideal choice for monitoring renal function damage and treatment outcomes, especially for chronic disease patients requiring long-term follow-up. Additionally, the real-time nature of ultrasound examinations allows for immediate assessment of dynamic changes in renal blood flow, which is of significant value in evaluating the immediate effects of treatments such as SKI [24,25].

The novelty of this study lies in investigating the specific application of ultrasound and CEUS in evaluating the efficacy of SKI in treating diabetic nephropathy, particularly in assessing renal function damage and changes in renal artery blood flow. This not only provides a reliable, non-invasive assessment tool but also offers scientific evidence for clinicians to optimize treatment strategies for diabetic nephropathy and improve patient prognosis. Through this research, we aimed to further validate the practicality and effectiveness of ultrasound and CEUS techniques in improving renal function assessment and monitoring treatment outcomes, thereby advancing the management of diabetic nephropathy.

MATERIALS AND METHODS

Subjects

A total of 124 DKD patients admitted to College of Traditional Chinese Medicine, Three Gorges University & Yichang Hospital of Traditional Chinese Medicine from January 2022 to January 2023 were recruited as patient group, while 26 healthy people who underwent physical examination in the same place during the same period were enrolled as the healthy group. The DKD patients were divided into observed subjects and controls by random number table method (n = 62). There were 30 male and 32 female patients in controls, averaging (62.25 ± 7.46) years old, with a disease course of (11.57 ± 3.24) years. Observed subjects included 31 males and 31 female patients, averaging (62.87 ± 7.63) years old, with a disease duration of (12.37 ± 2.63) years.

Inclusion criteria: patients diagnosed with DKD; patients having recent use of any treatment and medication; according to Mogensen staging, all patients were classified as clinical stage; patients with no history of allergy to the drugs adopted.

Exclusion criteria: patients with essential hypertension; Patients with renal injury caused by other diseases; patients with heart disease; patients who are pregnant or lactating.

This trial was approved by the ethics committee of the above-mentioned hospital (Registration number: XXX, registration date: XXX), and all enrolled patients signed informed consent.

Treatment protocols

The controls received conventional treatment, including oral hypoglycemic drugs and subcutaneous injection of insulin. The fasting blood glucose was

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controlled below 7.8 mM, and the postprandial blood glucose was controlled below 10 mM. The blood pressure was controlled under 130/mmHg with oral antihypertensive drugs. Oral lipid-lowering drugs called statins. The diet was mainly high protein, low fat, and low salt, and the patients were required to carry out appropriate exercise during the treatment.

All subjects were treated with SKI (20 mL × 5 pieces, National medicine permission number Z20040110, Xi 'an Shiji Shengkang Pharmaceutical Co., Ltd., China.) on the basis of conventional treatment. A total of 100 mL SKI was diluted with 200 mL 0.9% sodium chloride injection and intravenously infused once a day for 4 weeks.

Ultrasound diagnosis

All patients underwent ultrasound diagnosis before and after treatment to detect the renal function injury of patients. The GEi medical color ultrasound diagnostic instrument (VOLUSON S6, GE Company, USA) was used for ultrasound examination when the patient was in the supine position. First, the length, width, and thickness of the patient's kidney were detected. The detected data were put into the ellipsoid equation to calculate the kidney volume (V) and the ratio of V to body surface area (V/S). The status of internal renal arteries and blood flow changes were observed. The images were locally magnified at the locations of main renal artery (MRA), segmental renal artery (SRA), and interlobar renal artery (IRA). The sampling volume was set to 2-4 mm, and the angle was less than 60°. During the breath-holding period after inspiration, continuous recordings were made to measure the maximum diameter (D) of the vascular lumen at all levels of the artery. The system automatically calculated peak systolic velocity (PSV), end diastolic velocity (EDV), and resistance index (RI), pulse index (PI), and volumetric flow (VF) at all levels of artery. The upper, middle, and lower parts of the kidney were taken for detection when the blood flow changes of the kidney were measured. The final values were averaged to calculate the ratios of arterial blood flow at all levels of IRA/MRA-VF, IRA/SRA-VF, and SRA/MRA-VF.

The ellipsoid equation (1) is as follows.

$$V = {}^{\pi}/_{6} \times L \times W \times T \quad (1)$$

The equation (2) for body surface area is as follows.

$$S=0.0061\times H(cm)+0.0124\times weight(kg)-0.0099 \quad (2)$$

Diagnosis by CEUS

All patients underwent CEUS diagnosis before and after treatment, and the changes of renal artery blood flow were detected. The GEi medical color ultrasound diagnostic instrument VOLUSON S6 was used, and the contrast agent was perfluorobutane microspheres (GE Healthcare AS Company, USA) for injection. CEUS was performed in all patients in prone position with the probe placed on the back to view both kidneys. During the examination, the long axis of the kidney was used for observation, and the largest section was selected for observation. To keep the position and orientation of the probe stable, the patients were instructed to hold their breath. A total of 5 mL of normal saline (10 mL: 90 mg, National medicine permission number H20044024, Sinopharm Rongsheng Pharmaceutical Co., Ltd.) was injected into the vial of contrast medium and shaken evenly before the contrast medium was applied. Each time the contrast was performed, 1 mL of the contrast was drawn and injected through the cubital vein, followed by 5 mL of normal saline. The contrast agent was injected slowly, the image acquisition system was started, the acquisition sequence was set, and the continuous image acquisition was carried out. Changes in contrast medium intensity within the renal cortex were monitored for a duration of 3 minutes, capturing continuous changes following renal perfusion with the contrast medium. Following observation of one side of the kidney, a 15-minute interval was allowed before observing the other side. The ultrasound instrument was configured with the following settings: harmonic emission frequency of 8Hz, mechanical index of 0.07, depth focus set to 6 cm, and gain adjusted to 85%. Upon completion of the examination, all data were uniformly saved in DICOM format.

The time-intensity curve (TIC) of the contrast agent was fitted by the analysis software of VOLUSON S6 system. The region of interest (ROI) of the middle renal cortex was sampled, and the acoustic beam was vertical during the examination. This area had the same depth and position as the other ultrasound images of the patient, which was control area. Through γ fitting, the area under the curve (AUC), mean transit time (MTT), time to peak (TTP), peak intensity (PI), gradient between start frame to peak frame (Grad), and other parameters of blood flow changes can be obtained from the TIC.

Observation indicators

In the morning before ultrasound and CEUS, 5 mL of venous blood was orally drawn from the patient. Serum creatinine (Scr), β2-microglobulin (β2-MG), blood urea nitrogen (BUN), urinary albumin excretion (UAE), and hemorheological parameters were measured both before and after treatment. Additionally, blood routine and urine routine tests were conducted before and after treatment.

Statistical analysis

SPSS 22.0 (IBM Company, USA) was utilized for data analysis. Normally distributed measurement data were presented as mean ± standard deviation and analyzed by t-test. Non-normally distributed data

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were presented as median ± interquartile range and compared using nonparametric tests. Categorical data were denoted as percentages (%) and analyzed by chi-square test. Linear correlation analysis was employed to examine the relationship between TIC quantitative parameters and renal function indexes, with correlation coefficient (r) values calculated. Statistical significance was defined as $P < 0.05$.

RESULTS

Renal function indexes of patients

In figure 1, before treatment, neglectable differences ($P > 0.05$) existed between groups of patients in renal function indicators (Scr, β2-MG, BUN, and 24-hour UAE), indicating consistent baseline status among patients. After treatment, both groups of patients showed great decreases in Scr, β2-MG, BUN, and UAE levels relative to before treatment ($P < 0.05$), indicating effective improvement in renal function damage. The improvement in these indicators was more pronounced in observation group, suggesting that SKI may have a better recovery effect on renal function in patients with diabetic nephropathy.

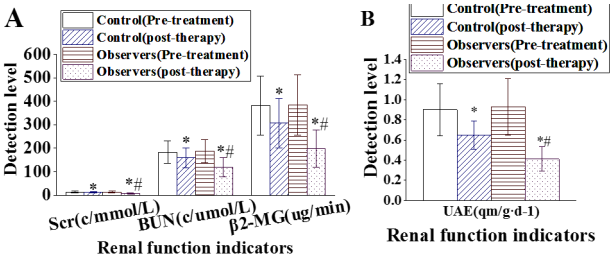

Figure 1. Comparison of renal function indicators in patients (A is Scr, β2-MG, BUN, B is UAE). *Note: *P < 0.05 vs. pre-remedy; #P < 0.05 vs. controls.*

Hemorheological indexes of patients

In figure 2, before treatment, no marked differences ($P > 0.05$) were found between groups of patients in PV, ESR, and fibrinogen levels, indicating similar hemorheological status at the beginning of the study. After treatment, both groups showed a drastic decrease in PV, ESR, and fibrinogen levels ($P < 0.05$), reflecting that the treatment helps improve hemorheological properties. The improvement in these indicators was more pronounced in observation group, suggesting that SKI may have advantages in improving the hemorheological status of patients with diabetic nephropathy.

Parameters of kidney volume and size in patients

In figure 3, after treatment, although there was a slight reduction in kidney length, width, and thickness in observation group, the differences were inconsiderable ($P > 0.05$), suggesting that SKI may have limited direct impact on renal parenchymal dimensions. However, the kidney volume and its ratio to body surface area (V/S) in observation group showed a prominent decrease relative to control group ($P < 0.05$). This implies the effectiveness of the treatment in reducing kidney volume and improving renal structural compactness.

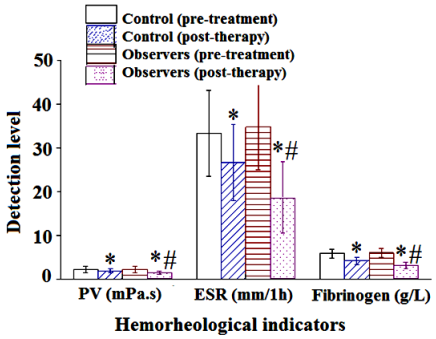

Figure 2. Contrast of hemorheological indexes in patients. Note: as against pre-remedy, *P < 0.05; as against controls, #P < 0.05.

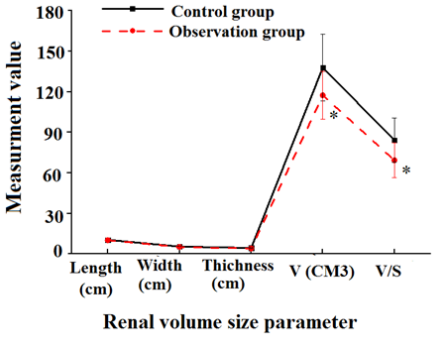

Figure 3. Contrast of renal volume and size parameters in patients. Note: *P < 0.05 as against controls.

Arterial blood flow parameters of patients

In figure 4, following treatment, in terms of blood flow parameters of the main renal artery, segmental arteries, and interlobar arteries (including PSV, EDV, RI, PI, and VF), observation group exhibited a substantial decrease relative to control group ($P < 0.05$). This demonstrates that SKI effectively improved renal arterial blood flow, reduced vascular resistance, and enhanced renal blood supply.

Parameters of renal artery blood flow ratio

In figure 5, after treatment, there were neglectable differences ($P > 0.05$) between groups of patients in the parameters of renal artery blood flow ratio (IRA/MRA-VF, IRA/SRA-VF, SRA/MRA-VF). This indicates that although SKI can improve blood flow parameters of renal artery, its effectiveness in adjusting the distribution of blood flow between different levels of renal arteries is limited.

Quantitative parameters of the TIC

In figure 6, following treatment, observation group demonstrated marked differences ($P < 0.05$) versus control group in the quantitative parameters of the TIC, including AUC, MTT, TTP, Grad, and PI. The observation group showed a marked decrease in AUC, MTT, TTP, and Grad values, accompanied by a marked

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increase in PI values. This indicates that SKI markedly improved renal blood perfusion status, bringing it closer to the blood flow dynamics of healthy individuals.

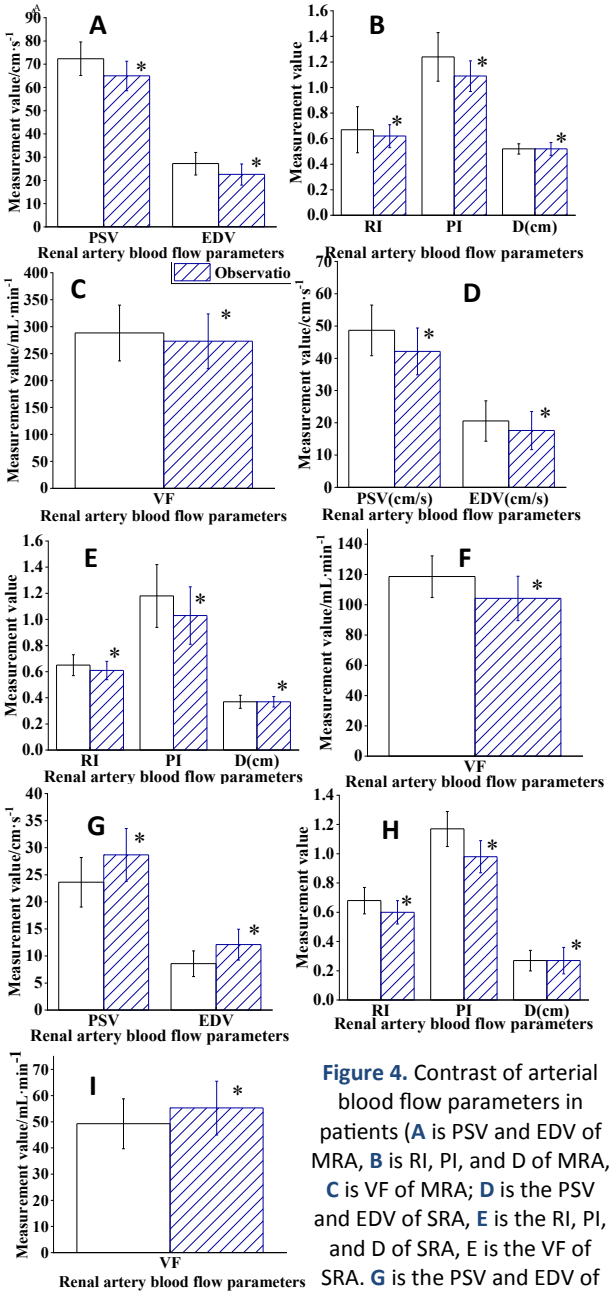

Figure 4. Contrast of arterial blood flow parameters in patients (**A** is PSV and EDV of MRA, **B** is RI, PI, and D of MRA, **C** is VF of MRA; **D** is the PSV and EDV of SRA, **E** is the RI, PI, and D of SRA, E is the VF of SRA. **G** is the PSV and EDV of IRA; **H** is the RI, PI, and D of IRA; **I** is the V of IRA). *Note: *P < 0.05 as against controls.*

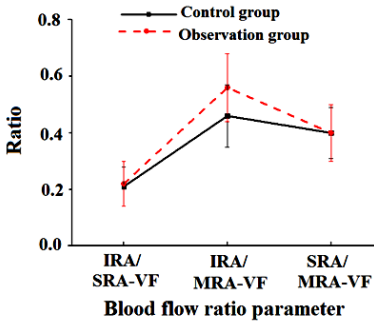

Figure 5. Contrast of parameters of renal artery blood flow ratio in sick persons.

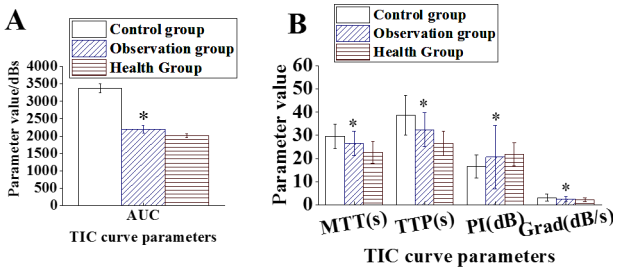

Figure 6. Contrast of TIC quantitative parameters in sick persons (A is AUC value, B is MTT, TTP, PI, Grad values). Note: as against controls, *P<0.05.

Relationship between renal function indexes and TIC quantitative parameters

In figure 7, the quantitative parameters of the TIC, including TTP, MTT, and Grad, exhibit a positive correlation with renal function indicators (UAE, Cr, BUN), whereas AUC and PI show a negative correlation with these renal function indicators. This finding revealed that TIC curve parameters can reflect renal function status, providing a new perspective and basis for monitoring renal function through CEUS techniques.

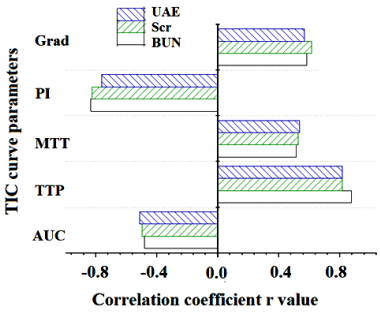

Figure 7. Contrast of the relationship between renal function indexes and TIC quantitative parameters in sick persons.

CEUS before and after treatment

In figures 8A and 8B below, in conventional mode ultrasound images, the morphology, size, and contour of the kidneys appeared generally normal, but there was enhanced echogenicity in the renal cortex, possibly indicating chronic changes. In the CEUS images, the density of light spots distributed within the kidneys indicated an increase in local blood flow, suggesting the occurrence of certain pathological conditions. Following treatment, there was improvement in renal blood flow, with good arterial perfusion, indicating that the treatment promotes renal blood circulation.

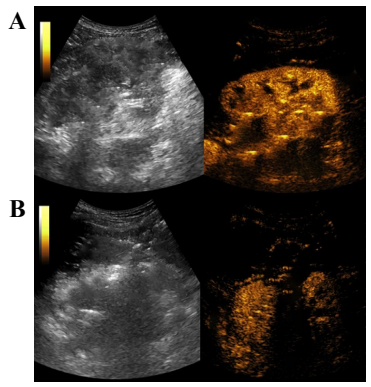

Figure 8. The CEUS before and after treatment in control group. *Note: Panel A represents before treatment, while panel B represents after treatment.*

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In figures 9A and 9B, the contour of the patient's kidneys was visible, but details were obscured by multiple hyperechoic areas, indicative of pathological changes within the kidneys. The boundary between the renal cortex and medulla appeared less distinct in some areas, suggesting structural changes due to pathology. After treatment, contrast-enhanced renal blood flow appeared as bright areas, indicating the distribution of the contrast agent within the kidneys and its utility in assessing blood flow status. The density and distribution of blood flow within the kidneys were more uniform, suggesting improved blood circulation.

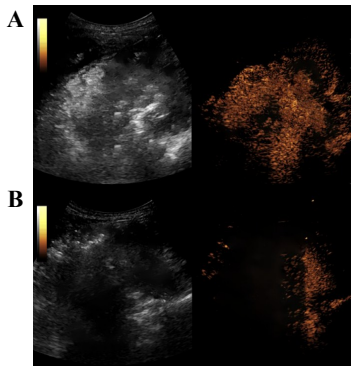

Figure 9. The CEUS before and after treatment in observation group. *Note: A represents before treatment, and B represents after treatment.*

DISCUSSION

Diabetic nephropathy is showing an increasing incidence trend along with the continuous rise in the number of diabetic patients. Its main pathological features include renal functional impairment and elevated urinary albumin, involving complex interactions among metabolic abnormalities, vascular lesions, and inflammatory processes [26]. This study comprehensively evaluated the clinical efficacy of SKI in the therapy of diabetic nephropathy, aiming to delve into its therapeutic mechanisms and explore the application value of ultrasound and CEUS techniques in monitoring treatment outcomes.

SKI contains various traditional Chinese medicine ingredients such as rhubarb, astragalus, salvia miltiorrhiza, and safflower, which synergistically intervene from multiple angles in the treatment of diabetic nephropathy [27]. Our study results demonstrated a marked reduction in SCr and BUN levels, along with a noticeable decrease in urinary albumin excretion rate after treatment (figure 1). This change indicates that SKI effectively improved renal function and reduced the renal burden. Rhubarb's diuretic and laxative effects can decrease the metabolic load on the kidneys, slowing the progression of glomerular interstitial fibrosis. Astragalus polysaccharides and other active components in astragalus enhance immune function, reduce endothelin levels, and improve renal microcirculation, thus protecting renal function. Salvia miltiorrhiza and safflower promote blood circulation, enhance renal blood supply, reduce inflammation levels, and further prevent exacerbation of renal damage. Wang *et al.* (2022) [28] studied the impact of SKI on CKD treatment, particularly how it inhibits the Wnt/β-catenin signaling to simultaneously target multiple renin-angiotensin system (RAS) genes, thus inhibiting CKD progression. Their study utilized an adenine-induced CKD rat model and angiotensin II-induced HK-2 and NRK-49F cell models to test the efficacy of SKI. The results indicated that SKI can inhibit renal function decline, hypertension, and renal fibrosis. Its mechanisms include reducing the protein expression of multiple RAS elements (such as angiotensin-converting enzyme and angiotensin II type 1 receptor), Wnt1, β-catenin, and their downstream target genes (such as Snail1, Twist, and matrix metalloproteinase-7). Additionally, emodin isolated from SKI can similarly inhibit renal function decline, epithelial-mesenchymal transition, and excessive activation of RAS and Wnt/β-catenin signaling pathways in these models. Compared with this study, Wang *et al.*'s [28] research also focuses on the efficacy of SKI in CKD treatment, showing marked similarity in treatment targets. However, Wang *et al.*'s [28] study points to new areas of action mechanisms for SKI, providing a broader perspective on its application in CKD treatment.

In this study, detailed assessments of renal morphology and hemodynamics were conducted using ultrasound and CEUS techniques. Following treatment, a marked reduction in renal volume and volume/surface area ratio was observed in the study group (figure 3), indicating that SKI effectively alleviated the issue of renal volume enlargement associated with diabetic nephropathy, possibly through its regulation of glomerular filtration rate and repair of tubular injury. Utilizing CEUS techniques, changes in renal blood flow were further examined. Significant improvements in quantitative parameters of the TIC, such as AUC, MTT, TTP, PI, and Grad (figure 6), not only demonstrate the positive impact of SKI on renal blood flow status but also reflect the improvement in renal microcirculation and restoration of glomerular filtration function. Additionally, the correlation analysis between TIC quantitative parameters and renal function indicators provides a novel perspective for understanding the pathological process of diabetic nephropathy and its response to treatment. Compared with the study by Zhu *et al.* (2022) [29] both studies focus on the application of SKI in the treatment of CKD. Their research evaluated the coagulation-fibrinolysis system and urinary protein, while our study evaluated renal functional damage and renal arterial hemodynamic changes using ultrasound and CEUS techniques, all aimed at exploring the effectiveness of SKI in the early treatment of CKD. Both studies demonstrated that

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SKI markedly improved physiological indicators associated with CKD. Their research indicated that SKI combined with Jinshuibao improved coagulation-fibrinolysis system indicators and reduced urinary protein excretion, whereas our study found that SKI improved renal hemodynamic status and mitigated renal functional damage. However, Zhu *et al.* [29] innovated in treatment regimens by attempting to enhance therapeutic effects through the combined use of different Chinese herbal medicines. The combination of these two studies may offer a more comprehensive perspective and strategy for the integrated treatment of diabetic nephropathy.

An in-depth analysis of the clinical efficacy of SKI in treating diabetic nephropathy was provided in this work, coupled with the application of ultrasound and CEUS techniques. It not only elucidates the mechanisms underlying the effects of SKI in improving renal function, regulating renal volume, and enhancing renal blood flow status but also demonstrates the marked value of ultrasound technology in monitoring the therapeutic effects of diabetic nephropathy. These findings hold important implications for optimizing the treatment strategies of diabetic nephropathy and improving patient prognosis. Future research should further explore the mechanisms of action of individual components within SKI and the broader application of ultrasound technology in early diagnosis and treatment monitoring of diabetic nephropathy, aiming to provide more precise and effective treatment regimens for patients with diabetic nephropathy.

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Conflict of interest: The authors declare no conflict of interest regarding the publication of this study.

Ethical considerations: This study was conducted in accordance with the principles outlined in the *Helsinki Declaration* and was approved by the Ethics Committees of the College of Traditional Chinese Medicine, Three Gorges University, and the Ethics Committee of Yichang Hospital of Traditional Chinese Medicine. Informed consent was obtained from all participants involved in the study.

Author contributions: S.S. conceptualized and designed the study, collected data, and drafted the manuscript. X.C. contributed to data analysis and interpretation, critically revised important intellectual content, and ensured the accuracy or completeness of various sections related to the literature. D.D. ensured appropriate investigation and resolution of issues related to the accuracy or completeness of the manuscript and served as the corresponding author.

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