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Value of Color Doppler Ultrasonography for Diagnosing Early Diabetic Nephropathy

Linshen Ke,[1†] Yanyan Guo,[2†] Xiuli Geng[3]

[1]Department of Ultrasound, Sanmen County People's Hospital, Sanmen 317100, Zhejiang Province, China
[2]Baota Bridge Community Health Service Center, Nanjing 210003, Jiangsu Province, China
[3]Department of Emergency, Qingdao Municipal Hospital, Qingdao 266000, Shandong Province, China
†The two authors contributed equally to this study.

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Original Paper

Introduction. Early diagnosis of diabetic nephropathy (DN), the leading cause of death in diabetic patients, is an important issue in preventing and reducing the disease burden for patients and the healthcare system. In this study, we aimed at investigating the value of color doppler ultrasonography in the diagnosis of early diabetic nephropathy (DN).

Methods. Two hundred and thirty-eight diabetic patients, were enrolled in this study and were categorized into, either control (n = 109) or study group (n = 129), according to 24 hours urinary albumin excretion rate (UAER), from January 2015 to March 2021. The morphologic findings of the kidneys were observed and compared, in both groups, by color doppler ultrasound technique, and blood flow of renal arteries was also measured, at all levels. Fasting plasma glucose (FPG), uric acid, homocysteine, beta-2-microglobulin, cystatin C, hemoglobin A1c (HbA1c) and CRP were also extracted from their laboratory results.

Results. Compared to the control group, the study group had lower intrarenal arterial end-diastolic blood flow velocity (EDV) and higher arterial resistance index (RI) ($P \sim < .05$). A significant diagnostic value of intrarenal arterial EDV and RI was found for early detection of DN ($P \sim < .05$). Intrarenal arterial RI and EDV showed positive correlations with UAER, FPG, uric acid, homocysteine, beta-2-microglobulin, cystatin C, HbA1c, and CRP ($P \sim < .05$).

Conclusion. Color doppler ultrasound markers of renal and intrarenal arteries has a high diagnostic value for DN at its early stage.

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INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of death in diabetic patients. Inappropriate treatment or uncontrolled diabetes has an irreversible impact on the kidneys, which eventually leads to kidney failure.[1] Diabetic nephropathy accounts for 50 percent of cases of kidney failure in China. The incidence of DN is increasing annually, and the disease tends to involve younger age groups, therefore requires more attention.[2] At present, it is well recognized that urinary albumin excretion rate (UAER) and beta-2-microglobulin level can be used for early diagnosis of DN. However, the pathological damage caused by DN might occur even before the appearance of albumin in the urine.[3] Therefore, timely treatment of diabetes could prevent such damage and is of great clinical significance.[4] Besides, it is difficult to diagnose early DN by laboratory tests alone, therefore additional paraclinical approaches may also be required.[5,6]

The development of ultrasound techniques helps in early diagnosis, and dynamic and continuous monitoring of the progression of DN.[7] Color doppler ultrasonography has been extensively used as a non-invasive, simple and rapid method to inspect many organs with a high diagnostic value.[8,9] However, this approach has not been used widely for the diagnosis of early DN, so far. The appropriate treatment of DN at its early stages can slow down or even reverse the progression of kidney damage.[10] Once clinical DN occurs, there is no effective treatment to stop its progression. Therefore, the early diagnosis of DN is of great importance. Mild albuminuria (UAER < 300 mg /24h) may remain undiagnosed and most DN patients are diagnosed only when they have overt albuminuria.[11] During this diagnostic gap, DN keeps progressing to its severe form, which is irreversible.

In this study, we aimed to investigate the value of color Doppler ultrasonography in the diagnosis of early diabetic nephropathy (DN), to improve prognosis, in patients with microalbuminuria.

MATERIALS AND METHODS

Subjects

Two hundred and thirty-eight diabetic patients were enrolled in this retrospective study, from January 2015 to March 2021. Diabetic patients aged more than 18 years old, with 24 hours UAER < 300 mg, without mental illness and a with complete laboratory data, were included. The exclusion criteria were history of other nondiabetic kidney diseases, urinary tract infection, severe multiorgan diseases, type 1 diabetes mellitus, malignant tumors, hematologic or autoimmune disorders and space-occupying lesions of the kidney. This study was approved by the hospital's ethics committee (approval No. QMH201501003), and all subjects signed informed written consents.

Measurement Methods

Color Doppler ultrasound device (EUB-8500, Hitachi Medical Corporation, Japan) with a probe frequency of 2.5 to 3.5 MHz, was used to visualize the kidneys. Patients were located in supine or lateral position, and the kidneys were observed, focusing on their morphology, structure and echogenicity. Then, the size of the kidneys (including longitudinal and transverse diameters and parenchymal thickness) was measured, as well. color Doppler flow imaging (CDFI), used to evaluate the blood flow of the main renal artery (MRA) at the renal hilum, interlobular renal arteries (IRA) and segmental renal arteries (SRA). Blood flow parameters including end-diastolic blood flow velocity (EDV) and peak systolic blood flow velocity (PSV), and the intrarenal arterial resistance index (RI) was calculated by the use of RI = (PSV - EDV) / PSV formula.

Observation Indicators

Demographic data including gender, age and body mass index (BMI), course of the disease, parity, gestational age and history of chronic diseases were recorded. Laboratory data including fasting plasma glucose, homocysteine level, β_2-microglobulin, serum creatinine, cystatin C, uric acid, blood urea nitrogen (BUN), UAER, hemoglobin A1c (HbA1c), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and C-reactive protein (CRP) were also extracted from the previous records. Color Doppler ultrasound measurement results were also recorded. Patients with UAER ≥ 30 mg /24h were included in the study group, and the control group included the patients without albuminuria.

Statistical Analysis

Statistical analysis was performed, using SPSS software version 20.0. Numerical data were expressed as percentage [n (%)], and intergroup comparisons were carried out by the chi-square test. Data with normal distribution were represented as mean ± standard deviation (`χ ± s), and intergroup comparisons were performed with the student's *t*-test. The ultrasound diagnostic criteria for DN were evaluated by plotting receiver operating characteristic (ROC) curves. Logistic regression was used to analyze the correlations between various factors and DN. Pearson's correlation test was applied to determine the correlations between arterial and arteriolar characteristics and UAER. $P < .05$ was considered statistically significant.

RESULTS

Data of Control and Research Groups

A total of 238 patients were categorized into two groups of control (n = 109, 45.80%) and study

groups (n = 129, 54.20%). No significant differences were found in age, gender, BMI and frequency of hypertension, coronary artery disease and hyperlipidemia, as well as the serum levels of triglyceride (TG), total cholesterol, HDL-C, LDL-C, creatinine, BUN and MRA-PSV, SRA-PSV and IRA-PSV between the two groups ($P > .05$).The two groups had significantly different courses of disease, fasting plasma glucose, uric acid, homocysteine, β_2-microglobulin, Cystatin C, HbA1c, CRP, MRA-EDV, MRA-RI, SRA-EDV, SRA-RI, IRA-EDV and IRA-RI ($P < .05$) (Table 1).

Color Doppler Ultrasound Images of Kidneys

Color Doppler ultrasound reports showed that, healthy individuals had broad bean-shaped kidneys, with smooth and intact capsule, uniform echogenicity and normal arterial blood flow. Patients in the control group had focal defects in the subcapsular vascular network, which prevented the color blood flow signal from reaching the subcapsular region. (Figure 1A). Intrarenal arterial blood flow signal was notably reduced and atypical dendritic distribution was seen in the IRA blood flow signal, in the study group (Figure 1B).

Logistic Regression Analysis Results of Early DN

The results of univariate logistic regression analysis revealed that the course of the disease, HDL-C, fasting plasma glucose, uric acid, homocysteine, β_2-microglobulin, Cystatin-C, HbA1c, CRP, MRA-EDV, MRA-RI, SRA-EDV, SRA-RI, IRA-EDV and IRA-RI were risk factors, associated with early DN. The results of multivariate logistic regression analysis demonstrated that fasting plasma glucose, uric acid, homocysteine,

Table 1. Data of Control and Research Groups ($\chi \pm s$)

Item	Control Group (n = 109)	Research Group (n = 129)	t/χ^2	*P*
Age, y	63.79 ± 5.21	65.33 ± 7.15	1.868	> .05
Gender, n (%)	56 (51.38)	63 (48.84)	0.152	> .05
BMI, kg/m^2	24.31 ± 2.63	24.08 ± 2.29	0.721	> .05
Course of Disease, y	5.20 ± 0.84	9.21 ± 1.45	25.480	< .001
Hypertension, n (%)	44 (40.37)	61 (47.29)	1.147	> .05
Coronary Heart Disease, n (%)	21 (19.27)	30 (23.26)	0.559	> .05
Hyperlipidemia, n (%)	62 (56.88)	88 (68.22)	3.258	> .05
Total Cholesterol, mmol/L	4.45 ± 1.02	4.74 ± 1.28	1.908	> .05
Triglycerides, mmol/L	1.49 ± 0.31	1.57 ± 0.33	1.916	> .05
LDL-C, mmol/L	2.74 ± 0.42	2.85 ± 0.48	1.864	> .05
HDL-C, mmol/L	1.06 ± 0.18	1.01 ± 0.21	1.952	> .05
Fasting plasma glucose, mmol/L	6.84 ± 0.85	7.58 ± 0.92	6.401	< .001
BUN, mmol/L	4.97 ± 1.08	5.24 ± 1.17	1.837	> .05
Serum Cr, μmol/L	73.18 ± 9.66	74.97 ± 10.12	1.388	> .05
Uric Acid, μmol/L	384.37 ± 41.90	446.29 ± 49.31	10.331	< .001
Homocysteine, μmol/L	16.15 ± 3.87	23.54 ± 5.09	12.423	< .001
β_2-Microglobuline, mg/L	5.49 ± 0.35	19.94 ± 1.72	86.189	< .001
Cystatin-C, mg/L	0.82 ± 0.27	1.63 ± 0.54	14.227	< .001
HbA1c (%)	6.16 ± 0.58	7.35 ± 0.87	12.174	< .001
CRP, mg/L	4.84 ± 1.14	7.36 ± 2.04	11.470	< .001
MRA-PSV, cm/s	76.24 ± 14.08	73.11 ± 13.29	1.762	> .05
MRA-EDV, cm/s	22.49 ± 4.16	15.82 ± 3.09	14.166	< .001
MRA-RI, cm/s	0.64 ± 0.06	0.72 ± 0.08	8.595	< .001
SRA-PSV, cm/s	53.14 ± 9.62	51.84 ± 9.23	1.062	> .05
SRA-EDV, cm/s	20.06 ± 4.19	14.23 ± 3.87	11.148	< .001
SRA-RI, cm/s	0.61 ± 0.04	0.70 ± 0.07	11.882	< .001
IRA-PSV, cm/s	36.42 ± 4.96	35.21 ± 5.41	1.785	> .05
IRA-EDV, cm/s	15.27 ± 2.33	10.12 ± 2.12	17.842	< .001
IRA-RI, cm/s	0.57 ± 0.03	0.68 ± 0.07	15.261	< .001

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; EDV, end-diastolic blood flow velocity; HbA1c, hemoglobin A1c; CRP, C-reactive protein; IRA, interlobular renal artery; MRA, main renal artery; PSV, peak systolic blood flow velocity; RI, intrarenal arterial blood flow resistance index; SRA, segmental renal artery

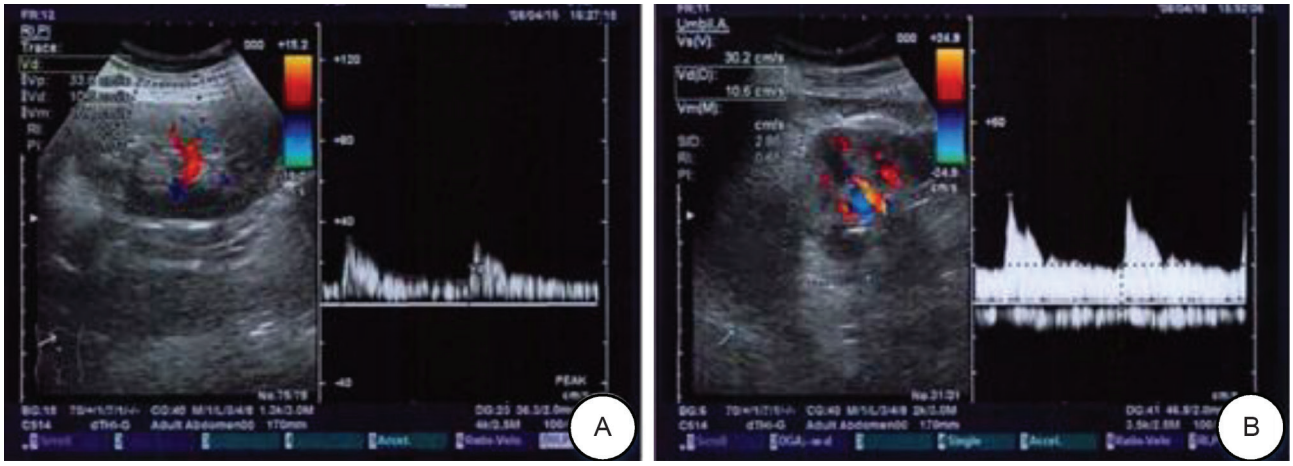

Figure 1. Color Doppler Ultrasound Images of the Kidneys (a: Control Group, b: Study Group)

β_2-microglobulin, Cystatin-C, HbA1c, CRP, MRA-EDV, MRA-RI, SRA-EDV, SRA-RI, IRA-EDV and IRA-RI were independent risk factors for early DN (Table 2).

Table 2. Logistic Regression Analysis Results of Early DN

Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, y	1.125 (0.996 to 1.255)	> .05		
Gender	0.986 (0.927 to 1.045)	> .05		
BMI, kg/m^2	0.974 (0.923 to 1.025)	> .05		
Course of Disease, y	1.794 (1.328 to 2.260)	< .001	1.290 (0.984 to 1.596)	> .05
Hypertension	1.098 (0.913 to 1.283)	> .05		
Coronary Artery Disease	1.107 (0.918 to 1.295)	> .05		
Hyperlipidemia	1.254 (0.983 to 1.525)	> .05		
Total Cholesterol, mmol/L	1.177 (0.986 to 1.368)	> .05		
Triglycerides, mmol/L	1.198 (0.990 to 1.406)	> .05		
LDL-C, mmol/L	1.085 (0.973 to 1.197)	> .05		
HDL-C, mmol/L	0.946 (0.898 to 0.995)	< .05	0.962 (0.901 to 1.022)	> .05
Fasting Plasma Glucose, mmol/L	1.528 (1.205 to 1.851)	< .001	1.461 (1.179 to 1.743)	< .001
BUN, mmol/L	1.059 (0.993 to 1.125)	> .05		
Serum Cr, μmol/L	1.136 (0.952 to 1.321)	> .05		
Uric Acid, μmol/L	1.851 (1.394 to 2.308)	< .001	1.695 (1.352 to 2.038)	< .001
Homocysteine, μmol/L	1.913 (1.481 to 2.344)	< .001	1.726 (1.529 to 1.923)	< .001
β_2-Microglobulin, mg/L	3.856 (2.139 to 5.573)	< .001	3.266 (2.549 to 3.983)	< .001
Cystatin-C, mg/L	2.058 (1.632 to 2.485)	< .001	1.859 (1.493 to 2.225)	< .001
HbA1c (%)	1.905 (1.473 to 2.337)	< .001	1.784 (1.408 to 2.160)	< .001
Hs-CRP, mg/L	1.883 (1.396 to 2.371)	< .001	1.799 (1.381 to 2.217)	< .001
MRA-PSV, cm/s	0.953 (0.894 to 1.012)	> .05		
MRA-EDV, cm/s	2.187 (1.650 to 2.725)	< .001	2.002 (1.573 to 2.431)	< .001
MRA-RI, cm/s	2.386 (1.893 to 2.879)	< .001	2.193 (1.751 to 2.635)	< .001
SRA-PSV, cm/s	0.969 (0.851 to 1.087)	> .05		
SRA-EDV, cm/s	1.805 (1.329 to 2.282)	< .001	1.746 (1.285 to 2.207)	< .001
SRA-RI, cm/s	1.974 (1.413 to 2.535)	< .001	1.837 (1.386 to 2.288)	< .001
IRA-PSV, cm/s	0.948 (0.884 to 1.011)	> .05		
IRA-EDV, cm/s	1.704 (1.377 to 2.032)	< .001	1.627 (1.285 to 1.969)	< .001
IRA-RI, cm/s	2.016 (1.597 to 2.436)	< .001	1.881 (1.520 to 2.243)	< .001

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; EDV, end-diastolic blood flow velocity; HbA1c, hemoglobin A1c; CRP, hypersensitive C-reactive protein; IRA, interlobular renal artery; MRA, main renal artery; PSV, peak systolic blood flow velocity; RI, intrarenal arterial blood flow resistance index; Scr, serum creatinine; SRA, segmental renal artery

ROC Curves of Color Doppler Ultrasound Indicators for Diagnosing Early DN

The ROC curves of color Doppler ultrasound markers for diagnosis of DN at its early stage were plotted (Figure 2). The results revealed that the area under the curve (AUC) of MRA-RI, MRA-EDV, SRA-RI, SRA-EDV, IRA-RI, and IRA-EDV in the diagnosis of DN were 0.847 (0.833 to 0.861), 0.791 (0.776 to 0.806), 0.820 (0.805 to 0.835), 0.758 (0.743 to 0.773), 0.842 (0.828 to 0.857), and 0.746 (0.730 to 0.762); respectively, indicating significant diagnostic values (Table 3).

Correlation Analysis Results of Color Doppler Ultrasound Indicators and Clinical Data

Pearson's correlation analysis showed that color Doppler ultrasonographic markers including MRA-EDV, SRA-EDV, and IRA-EDV were negatively associated with UAER, fasting plasma glucose, uric acid, homocysteine, β_2-microglobulin, Cystatin-C, HbA1c, and CRP ($P < .05$); while MRA-RI, SRA-RI, and IRA-RI were positively related to these laboratory data ($P < .05$) (Table 4).

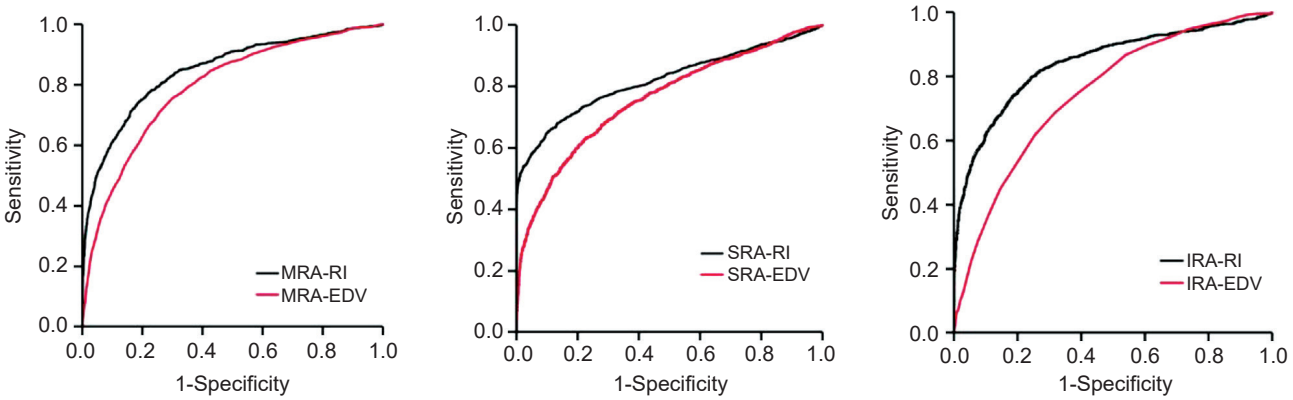

Figure 2. ROC Curves of Color Doppler Ultrasound Markers for Diagnosis of Early DN

Table 3. Diagnostic Efficacy of Color Doppler Ultrasound for Rarly DN Determined by ROC Curves

Variable	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Youden's Index	*P*
MRA-RI	0.847 (0.833 to 0.861)	74.42	81.22	0.5564	< .001
MRA-EDV	0.791 (0.776 to 0.806)	75.67	69.85	0.4552	< .001
SRA-RI	0.820 (0.805 to 0.835)	64.88	90.05	0.5493	< .001
SRA-EDV	0.758 (0.743 to 0.773)	63.26	77.15	0.4041	< .001
IRA-RI	0.842 (0.828 to 0.857)	73.79	81.69	0.5548	< .001
IRA-EDV	0.746 (0.730 to 0.762)	68.78	68.14	0.3692	< .001

Table 4. Correlation Analysis Results of Color Doppler Ultrasound Indicators and Clinical Data

Variable	UAER	Fasting Blood Glucose	UA	Hcy	β_2-MG	Cys-C	HbA1c	CRP
MRA-RI	0.656*	0.328*	0.400*	0.418*	0.604*	0.454*	0.442*	0.417*
MRA-EDV	-0.584*	0.215*	-0.288*	-0.307*	-0.526*	-0.325*	-0.336*	-0.296*
SRA-RI	0.625*	0.304*	0.406*	0.402*	0.581*	0.433*	0.417*	0.438*
SRA-EDV	-0.562*	0.198*	-0.239*	-0.299*	-0.515*	-0.349*	-0.310*	-0.281*
IRA-RI	0.601*	0.273*	0.371*	0.381*	0.557*	0.435*	0.392*	0.381*
IRA-EDV	-0.543*	0.165*	-0.207*	-0.277*	-0.493*	-0.291*	-0.283*	-0.255*

*$P < .05$

Abbreviations: β_2-MG, beta-2-microglobulin; Cys-C, cystatin C; EDV, end-diastolic blood flow velocity; HbA1c, hemoglobin A1c; Hcy, homocysteine; hs-CRP, hypersensitive C-reactive protein; IRA, interlobular renal artery; MRA, main renal artery at renal hilum; RI, intrarenal arterial blood flow resistance index; SRA, segmental renal artery; UA, uric acid.

DISCUSSION

In diabetic patients, the osmotic effect of persistent hyperglycemia can cause glomerular enlargement and glomerular hyperfiltration, which can damage glomerular capillary endothelial cells, increase mesangial matrix and eventually lead to glomerulosclerosis. As a result, atherosclerosis occurs in renal arteries and their branches, and albumin appears in the urine.[12,13] Moreover, cytokine levels alter as a result of abnormal metabolism in diabetic individuals, which stimulates the production of extracellular matrix, impedes its degradation, and finally causes accumulation of extracellular matrix. All these events induce renal tissue fibrosis and kidney damage and result in the development of DN.[14] The entire pathological process will affect the patient's hemodynamics, and blood flow resistance will increase markedly.[15] In a healthy human, the blood flow velocity curve of the renal arteries is characterized by low diastolic blood flow resistance, which keeps the blood flow moving forward, and high systolic blood flow resistance, which increases the blood flow velocity.[16] In contrast, in patients with DN, the gradual thickening of the glomerular capillary basement membrane and obstruction of glomerular capillary lumens lead to hemodynamic changes, which increase the resistance to the forward flow of blood. With the progression of the disease, hemodynamic changes would also progress to renal arteries at all levels.[17] Therefore, observing the hemodynamic changes of renal arteries in diabetic patients, helps in the evaluation and identification of DN in diabetic patients.

Color Doppler ultrasonography is a non-invasive method for evaluating fluctuations in renal blood flow. It can directly depict the morphological changes of the kidneys. In addition, the blood flow can be studied, in renal arteries by calculating the relevant blood flow parameters, such as PSV and EDV and RI. Peak systolic velocity (PSV) is used to determine renal vascular filling and blood supply, end-diastolic velocity (EDV) can reflect the blood flow in the renal arteries and resistant index (RI) is closely related to the elasticity of the renal blood vessels, which can reflect the resistance in the vascular bed.[18] This research also investigated renal arteries using MRA, SRA, and IRA blood flow parameters, which can accurately reflect the blood flow in renal arteries at all levels, as well as changes in kidney function. The results demonstrated that in the control group renal blood flow had low resistance and high flow velocity, and the blood flow signal was distributed along the MRAs, SRAs, IRAs and arcuate arteries, manifested by typical dendritic distribution on the blood flow images. These findings indicate that there is no significant lesion in the intrarenal arteries and glomerular capillaries in diabetic patients without DN. In contrast, in the study group, the renal blood flow had high resistance and low flow velocity. The arterial wall thickness was irregular, blood flow signals were prominently reduced or even oscillatory, and vascular sclerosis and intimal lesions were apparent in renal arteries, at all levels These changes lead to poor elasticity of the vascular wall and narrowing or even obstruction of their lumens, which further affect the blood supply to the kidneys and tissue damage. The results of renal color Doppler ultrasonography showed that MRA-EDV, SRA-EDV and IRA-EDV values were significantly lower in the study group than in the control group, while the study group had higher RI values in MRA, SRA and IRA, as compared to the control group, indicating low flow velocity and high resistance in the study group. In addition, ROC curve analysis demonstrated specific diagnostic values for the aforementioned six DN indicators, suggesting that DN might be diagnosed at its early stage, using color Doppler ultrasonography.

The results of the present study also revealed that MRA-EDV, SRA-EDV, and IRA-EDV values were negatively associated, while MRA-RI, SRA-RI, and IRA-RI values were positively associated, with UAER, fasting plasma glucose, uric acid, homocysteine, β_2-microglobulin, Cystatin-C, HbA1c, and CRP ($P < .05$). As was already mentioned, the osmotic effect of hyperglycemia can cause glomerular enlargement and hyperfiltration, and damage kidneys to induce atherosclerosis and microalbuminuria. Therefore, fasting plasma glucose, HbA1c and UAER are correlated with ultrasound markers.[19] High homocysteine levels can initiate glomerular endothelial cell death, through membrane lipid peroxidation, and loss of cell integrity, resulting in impaired glomerular function and increased intrarenal arterial resistance.[20] Moreover, β_2-microglobulin, Cystatin-C, uric acid, and CRP are all sensitive markers of early

kidney impairment.[21-23] The correlation between the abovementioned parameters and color Doppler ultrasound markers further confirmed that color Doppler ultrasonography can be utilized for early diagnosis of DN.

CONCLUSION

In conclusion, compared to diabetic patients without nephropathy, diabetic patients with DN exhibit increased renal vascular resistance and decreased blood flow velocity. Color Doppler ultrasound can display the changes in renal artery blood flow, which helps in early diagnosis and treatment of DN, before obvious clinical abnormalities of kidney function appear, and assist to control the kidney damage before the occurrence of permanent changes, thus reducing the impact of DN and improving the prognosis in diabetic patients.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Correspondence to:
Xiuli Geng, MD
Department of Emergency, Qingdao Municipal Hospital, Qingdao 266000, Shandong Province, China
E-mail: gengxlqmh@dh-edu.cn

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