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FULL PAPER

Non-invasive assessment of early stage diabetic nephropathy by DTI and BOLD MRI

¹YOU-ZHEN FENG, MS, ¹YAO-JIANG YE, BS, ¹ZHONG-YUAN CHENG, MS, ¹JUN-JIAO HU, BS,
²CHUANG-BIAO ZHANG, MS, ³LONG QIAN, PHD, ²XIAO-HUA LU, MD and ¹XIANG-RAN CAI, MD

¹Medical Imaging Center, First Affiliated Hospital, Jinan University, Guangzhou, Guangdong, China

²Endocrinology department, First Affiliated Hospital, Jinan University, Guangzhou, Guangdong, China

³GE Healthcare, Beijing, China

Address correspondence to: Mrs Xiang-Ran Cai

E-mail: caixran@jnu.edu.cn

The authors Xiao-Hua Lu and Xiang-Ran Cai contributed equally to the work.

The authors You-Zhen Feng and Yao-Jiang Ye contributed equally to the work.

Objective: Patients with diabetes mellitus, diabetic nephropathy (DN) and healthy donor were analyzed to test whether the early DN patients can be detected using both blood oxygenation level dependent (BOLD) and diffusion tensor imaging.

Methods: This study was approved by the Ethics Committee of our hospital. MR images were acquired on a 3.0-Tesla MR system (Discovery MR750, General Electric, Milwaukee, WI). 30 diabetic patients were divided into NAU (normal to mildly increased albuminuria, $N = 15$) and MAU (moderately increased albuminuria, $N = 15$) group based on the absence or presence of microalbuminuria. 15 controls with sex- and age-matched were enrolled in the study. Prior to MRI scan, all participants were instructed to collect their fresh morning urine samples for quantitative measurement of urinary microalbumin and urinary creatinine. Then, the estimations of serum creatinine, serum uric acid, HbA1c and fasting plasma glucose as well as fundus examinations were performed in all subjects. Then, the values of albumin-creatinine ratio (ACR) and estimated glomerular filtration rate were also calculated. All subjects underwent renal diffusion tensor imaging (DTI) and BOLD acquisition after fasting for 4 h. Regions of interest were placed in renal medulla and cortex for evaluating apparent diffusion coefficient (ADC), fractional anisotropy (FA) and $R2^*$ values by two experienced radiologists. The consistency between the two observations was estimated using intragroup correlation coefficients. To test differences in ADC, FA and $R2^*$ values across the three groups, the data were analyzed using separate one-way ANOVAs.

Post-hoc pair wise comparisons were then performed using t -test. To investigate the clinical relevance of imaging parameters in both regions across the three groups, the correlations of values of the ACR/estimated glomerular filtration rate and of the ADC/FA/ $R2^*$ were calculated.

Results: There was a high level of consistency of those ADC, FA and $R2^*$ values across the three groups on both renal cortex and medulla measured by the two doctors. The FA value of medulla in MAU group was lower than that in control ($p < 0.01$). The $R2^*$ value of medulla in the NAU group was higher than that in the control ($p < 0.01$), and the $R2^*$ value of medulla in the MAU group was lower than that in the control ($p = 0.009$). Moreover, the current study revealed a decreasing trend in FA values of the renal medulla from the control group to NAU and MAU groups. Finally, a weak negatively correlation between medullary $R2^*$ and ACR was found in current study.

Conclusion: Medullary $R2^*$ value might be a new more sensitive predictor of early DN. Meanwhile, BOLD imaging detected the medullary hypoxia at the simply diabetic stage, while DTI didn't identify the medullary directional diffusion changes at this stage. Based on our assumption mentioned above, it's presumable that BOLD imaging may be more sensitive for assessment of the early renal function changes than DTI. These imaging techniques are more accurate and practical than conventional tests.

Advances in knowledge: Non-invasive MRI was used to detect renal function changes at early DN stage.

INTRODUCTION

Diabetes mellitus (DM) is a major cause of morbidity and mortality worldwide. In China, its incidence has dramatically increased, and it has become a major public health crisis in recent decades due to population growth, ageing, and urbanization. Some researchers predict that China will soon become the country with the highest prevalence of diabetes in the world.¹ The most serious consequence of DM is that the persistent state of hyperglycaemia leads to pathological changes in various organs of the human body. Among them, chronic progressive kidney damage is one of the main complications.² At present, the clinical diagnosis of diabetic nephropathy (DN) relies mainly on the detection of urine microalbumin. However, in the early stage of kidney damage, the compensatory function of the kidney itself often makes the result of this test negative.³ Renal biopsy is another modality to detect the early DN, but it cannot be widely used for the early diagnosis of DN in clinical practice because of its invasiveness. Therefore, it is urgent for us to search for other effective methods to diagnose and assess the early stage of kidney damage. In addition, as for the pathogenesis of DN, although it has not yet been clearly elucidated,⁴ the increasing number of studies show that long-term chronic hypoxia plays a leading role in the pathophysiological changes of DN, and the changes of hypoxia can be observed in the early stage of DN.^{5–8} It has been found that a common pathway to nephropathy is the development of kidney hypoxia.⁸

In the past decades, blood oxygenation level dependent (BOLD) imaging uses deoxygenated haemoglobin as an endogenous contrast agent to perform non-invasive assessment of tissue oxygenation levels. Additionally, the previous studies^{6,9–11} demonstrated that diffusion imaging may account for the tissue damage typically induced by acute or chronic hypoxia. Diffusion tensor imaging (DTI) can display how water travels along multiple directions in kidney.⁹ Many studies have shown that functional magnetic resonance imaging (fMRI), including BOLD imaging and DTI, are safe, non-invasive imaging modalities capable of providing quantitative parameters to assess the change of renal microstructure and function. In addition, our previous study and others have demonstrated that some fMRI techniques could be more sensitive than albuminuria to detect early-stage DN.^{10,11} Yet, there are only a limited number of studies concerning the use of renal quantitative BOLD imaging and DTI in early DN in humans.^{11–13} Hence, our aim in this study was to assess the capability of quantitative BOLD imaging and DTI to detect early DN.

METHODS AND MATERIALS

Study population

This study was approved by the Ethics Committee of our hospital. All participants signed informed consent. Declarations of interest: none.

A total of 33 patients with Type two diabetes was enrolled from January 2015 to February 2017. The patients were admitted to the Department of Endocrinology in our hospital. The specific inclusion criteria were as follows: (1) age of 18 to 80 years; (2) with type II diabetes, per the 2007 American Diabetes Association diagnosis; (3) no other diseases affecting kidney function (e.g., gout, blood disorders, essential hypertension, nephrotic syndrome); (4) no

receiving any medication that may impact upon renal blood flow; and (5) no MRI examination contraindications. From this group, three cases were excluded due to the following reasons: (1) poor image quality seriously affecting the measurements ($n = 1$) and (2) large (>2 cm) or multiple (more than 3) simple kidney cysts, hamartomas (>0.5 cm), or other lesions within the kidney ($n = 2$). The diabetic group was divided into the NAU group [normal to mildly increased albuminuria, <30 mg/g for albumin–creatinine ratio (ACR)] and the MAU group (moderately increased albuminuria, $30–300$ mg/g for ACR) according to the ACR test results. Ultimately, 30 cases were included in this study.

Fifteen healthy volunteers, who underwent physical examination in our hospital from January 2015 to February 2017, were included according to the specific criteria: (1) 18 to 80 years of age; (2) no hypertension, diabetes, and other diseases; (3) no other systemic metabolic diseases that affect renal function (e.g., gout, blood disorders, essential hypertension, nephrotic syndrome); and (4) no contraindication to MRI.

In this study, the National Kidney Foundation (NKF) and Food and Drug Administration (FDA) workshops (2009) were used to classify albuminuria.¹⁴ For those who have been diagnosed with Type two diabetes and have had retinopathy for more than 5 years, when the ACR test is performed (after excluding other causes of proteinuria) three times in 6 months to the aforementioned range, diabetic proteinuria can be considered. The patients with diabetes were stratified into two groups based on albuminuria category: NAU ($ACR < 30$ mg/g) and MAU ($30 \leq ACR \leq 300$ mg/g).

Study protocol

The MRI was performed on the GE HC 3.0T Discovery MR750 superconductor scanner with an 8-channel body coil and post-processing workstation (Sun Advantage Workstation 4.5, SDW 4.5). Prior to the MRI scan, all the subjects fasted for 4 h, and the water intake did not exceed 350 ml within 2 h. The subjects were then scanned in the supine position with a breathing belt tied in the middle abdomen.

Scan sequence and parameters

Coronal DTI images with respiratory triggering were performed with a diffusion gradient oriented in 16 directions, b-values (0 s/ mm^2 , 600 s/ mm^2), using SE/EPI sequence: TR: 6667 ms; TE: 51.8 ms; layer thickness: 5.0 mm; layer interval: 1.0 mm; FOV = 40.0×32 cm; bandwidth: 250 kHz; matrix: 128×160 ; NEX: 1.0; acquisition time: 2 min and 27 sec.

Coronal BOLD images were obtained in inspiration and 8T2*-weighted images for each coronal slice and were recorded within a single breathhold of 15 s with a modified multi echo data image combination sequence (MEDIC) for BOLD analysis with the following parameters: eight echoes, TR: 150 ms; TE: 3.4–27.8 ms; layer thickness: 5.0 mm; layer interval: 1.0 mm; flip angle 30° ; FOV = 40.0×32 cm; bandwidth: 50 kHz; matrix: 256×192 ; NEX: 1.0; acquisition time: 45–52 s. Each set of eight T2*-weighted images, corresponding to eight different echoes, was acquired during a 15 sec each breath hold.

Figure 1. Example of ROI measurement on the coronal BOLD image (TE = 27.8 ms, (A1) and DTI (with $b = 0$ s/mm², (B1) in a 43-year-old female. ROIs were positioned in the medulla (yellow) and cortex (blue) in the upper, middle, and lower parts of each kidney. The ROI had an area of 22–200 mm² (medulla: 50–200 mm²; cortex: 22–40 mm²). The pseudocolor images are R2* map (A2), T2* map (A3), FA map (B2), and ADC map (B3). ADC, apparent diffusion coefficient; BOLD, blood oxygenation level dependent; DTI, diffusion tensor imaging; FA, fractional anisotropy; ROI, region of interest.

Image analysis

The morphological evaluation of all patients' kidneys was performed on coronal DTI ($b = 0$) image and T2* map. Considering that the right kidney was affected less by the great vessels of the heart and diaphragmatic movements, measurement of data was in the right kidney. The evaluation was performed independently by two physicians with 5 and 17 years' experience with abdominal imaging diagnosis. The first step was to record whether the kidneys had cysts or other space occupying lesion. When there was a disagreement, the result of the negotiation was decided. Second, the two individuals completed the measurement of DTI and BOLD data independently, and were blinded to the population group before the measurement. On the b_0 and T2* maps, the demarcation of the renal cortex and medulla is clear. Regions of interest (ROIs) with a unfixed size of 650 to 850 mm² and 22 to 40 mm² were placed into medulla and cortex of the right kidney on apparent diffusion coefficient (ADC), fractional anisotropy (FA), and T2* maps (Figure 1). Manual measurement was used on renal cortical region of interest (ROI) to cover the entire renal cortex while avoiding blood vessels, renal pelvis, and the kidney edge as much as possible. For the renal medulla, three ROIs were placed in the upper, middle, and lower poles, respectively. The measurements were conducted three times, and the average values were used for statistical analysis. Results were expressed as the means $\pm$ standard deviation.

Laboratory inspection

Prior to the MRI scan, all participants took fresh morning urine for urinary microalbumin and quantitative measurement of urinary creatinine to avoid strenuous exercise, fever, pregnancy, or lactation. Serum creatinine, serum uric acid, HbA1c, fasting plasma glucose, and fundus examinations were performed on all participants. In addition, we calculated the ACR and estimated the glomerular filtration rate (eGFR) according to the Chinese dietary modification of dietary disease (MDRD) equation^{15,16}:

$$\text{eGFR (mL/min/1.73 m}^2\text{)} = 175 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ female})$$

Statistical analysis

The statistical analysis was performed using SPSS 20.0 (IBM, Corp. Armonk, NY) statistical software package, and statistical significance was set at $p < 0.05$. Parameters were expressed as mean $\pm$ standard deviation. Two radiologists measured the ADC value, FA value, and T2* value using the intraclass correlation coefficient (ICC) for consistency testing, as follows: ICC < 0.1 , no consistency; $0.11 < \text{ICC} < 0.4$, lower agreement; $0.41 < \text{ICC} < 0.6$, consistent; $0.61 < \text{ICC} < 0.80$, moderate agreement; and ICC > 0.8 , consistency is very good. The consistency testing performed with two-way mixed models, single measures used, ICCs reported for consistency agreement. The three groups of subjects were compared for sex, age, ACR, and eGFR. Male-to-female ration of three groups was compared using χ^2 test. Age, ACR, eGFR and the mean renal cortical and medullary BOLD and DTI parameters among three groups were analyzed using separate one-way ANOVAs. Post-hoc multiple pairwise comparisons were performed with the Bonferroni or Tamhane's T2 test. Pearson correlation analysis was used to correlate the BOLD and DTI parameter values with ACR and eGFR.

RESULTS

General clinical data and laboratory test results of the subjects

The age, sex distribution, ACR, eGFR and HbA1c values of the three groups are shown in Table 1. The difference of gender and age among the three groups was not significant, and there was no statistically significant difference (p values were 1.000 and 0.362, respectively). There was no difference in urinary microalbumin values between the control and the NAU groups ($p = 0.962$), whereas the urine microalbumin in the MAU group was significantly higher than those in the other two groups ($p = 0.000$). There was no significant difference in eGFR among the three groups ($p = 0.137$).

Table 1. Characteristics of the patient groups

	Control (<i>n</i> = 15)	NAU (<i>n</i> = 15)	MAU (<i>n</i> = 15)	F	^a <i>p</i>	^b <i>p</i>	^c <i>p</i>	^d <i>p</i>
Sex (Male/Female)	5/10	8/7	5/10		1.000			
Age (y)	50.80 ± 8.05	52.33 ± 8.65	56.00 ± 13.00	1.042	0.362			
ACR (mg/g)	5.78 ± 3.12	7.02 ± 3.28	72.20 ± 150.00	32.206	0.000	0.962 ^c	0.000 ^c	0.000 ^c
eGFR (mL/min/1.73 m ²)	103.65 ± 11.9	102.02 ± 17.80	97.87 ± 8.83	2.088	0.137			
HbA1c	4.86 ± 0.16	8.79 ± 1.90	8.05 ± 2.21	22.138	0.000	0.705 ^c	0.000 ^c	0.000 ^c

ACR, albumin-creatinine ratio; MAU, moderately increased albuminuria; NAU, normal to mildly increased albuminuria; SD, standard deviation; eGFR, estimated glomerular filtration rate.

The sex distribution, age, ACR, and eGFR values of the three group. ACR, (mg/g) ; eGFR (mL/min/1.73 m²; Control group, healthy volunteers; NAU group <30 mg/g for ACR; MAU group 30–300 mg/g for ACR. Results were expressed as the means ± SD (*n* = 15). Male-to-female ration of three groups was compared using χ^2 test. *p* < 0.05 was considered significant.

^aAge, ACR and eGFR between the groups were compared using the One-way ANOVA test.

^bPost-hoc paired comparisons between the MAU group and the NAU group;

^cPost-hoc paired comparisons between the NAU group and the control group;

^dPost-hoc paired comparisons between the MAU group and the control group ;

^ePost-hoc paired comparisons between groups using the Tamhane's T2 test.

ICC analysis of the two researchers' measurement data was done, and ICC analysis was used to compare the results of the two observers to the three group data. The consistency of the ADC values, FA values, and the rate of spin dephasing (R2*) values of the renal cortex and medulla of the three groups measured by the two physicians was very good (Table 2).

Comparison of ADC values, FA values, and R2* values of the cortex and medulla between the three groups

The FA value of the medulla in the MAU group was lower than that in the control group (*p* = 0.010). The R2* value of the medulla in the NAU group was higher than that in the control group (*p* = 0.000), and the R2* value of the medulla in the MAU group was lower than that in the NAU group (*p* = 0.000). There was no statistical difference in cortical ADC values, FA values, and R2* values among the three groups (Table 3, Figure 2).

Correlative analysis of the three groups of the cortex and medulla ADC values, FA values, and R2* values and eGFR, ACR and HbA1c

Pearson correlation analysis was used to analyze the relationship among the cortex and medulla ADC, FA, and R2* values, and ACR and eGFR (Figure 3).

Figure 3 and Supplementary Table 1 shows that the R2* value of the medulla in the control group has a weak correlation with ACR, and the other parameters had no correlation with ACR, eGFR or HbA1c.

DISCUSSION

In the current study, we evaluated the sensitivity of BOLD imaging and DTI assessments to non-invasively detect renal functional differences in diabetic patients with or without microalbuminuria compared with healthy control subjects. Our results demonstrated the significantly greater R2* value in the renal medulla in diabetic patients without proteinuria, indicative of medullary hypoxia. Although the medullary FA value had a decreasing trend across the three groups, the significant decrease was found only in diabetic patients with microalbuminuria, suggestive of medullary microstructure alterations in renal tubules or vessels. These MRI findings suggest that BOLD imaging and DTI can provide a non-invasive assessment of hypoxia and regional renal microstructural changes in the early stage of DN. BOLD imaging is novel fMRI technique using the paramagnetic properties of deoxy-haemoglobin to acquire images sensitive to local tissue oxygen concentration. It has been extensively used for oxygenation measurements in renal diseases because intrarenal oxygenation is an important determinant in renal pathophysiology.^{6,7,17–23}

Table 2. Intraclass correlation analysis of data measured by two observers

	ADC (<i>n</i> = 45)		FA (<i>n</i> = 45)		R2* (<i>n</i> = 45)	
	ICC	95% CI	ICC	95% CI	ICC	95% CI
Cortex	0.886	0.802–0.936	0.992	0.985–0.995	0.938	0.891–0.966
Medulla	0.907	0.837–0.948	0.940	0.894–0.967	0.965	0.936–0.980

ADC, apparent diffusion coefficient; CI, confidence interval; FA, fractional anisotropy; ICC, intraclass correlation coefficient; R2*, rate of spin dephasing.

Intraclass correlation analysis of data measured by twodoctors. Results were expressed as the ICC, 95% SD (*n* = 45).

Table 3. Comparisons of ADC values, FA values, and R2* values of cortex and medulla among the three groups

		Control (n = 15)	NAU (n = 15)	MAU (n = 15)	F	^a p	^b p	^c p	^d p
Cortex	ADC (×10 ⁻³)	2.11 ± 0.18	2.02 ± 0.40	2.04 ± 0.10	2.164	0.127			
	FA	0.17 ± 0.03	0.17 ± 0.03	0.16 ± 0.03	0.480	0.622			
	R2* ms ⁻¹	16.66 ± 1.63	16.43 ± 2.12	17.03 ± 1.85	0.387	0.681			
Medulla	ADC (×10 ⁻³)	1.83 ± 0.21	1.77 ± 0.17	1.88 ± 0.15	1.669	0.201			
	FA ^e	0.29 ± 0.03	0.27 ± 0.04	0.26 ± 0.02	3.997	0.026	0.791	0.296	0.010
	R2* ms ^{-1f}	28.85 ± 3.76	34.61 ± 3.39	28.14 ± 3.76	14.268	0.000	0.000	0.000	0.940

ADC, apparent diffusion coefficient; FA, fractional anisotropy; R2*, rate of spin dephasing.

Data are represented as means ± standard deviation. Comparisons of ADC values, FA values, and R2* values of cortex and medulla among the three groups. ADC (×10⁻³ mm²/s) ; FA; R2* (ms⁻¹). Results were expressed as the means ± SD (n = 15). *p* < 0.05 was considered significant.

^aMean renal cortical and medullary BOLD and DTI parameters among three groups were analyzed using separate one-way ANOVAs;

^bPost-hoc paired comparisons between the MAU group and the NAU group;

^cPost-hoc paired comparisons between the NAU group and the control group;

^dPost-hoc paired comparisons between the MAU group and the control group;

^ePost-hoc paired comparisons between groups using the Tamhane's T2 test.

^fPost-hoc paired comparisons between groups using the Bonferroni test;

Interestingly, in the present study, the medullary R2* value in diabetic patients with normoalbuminuria was significantly greater than those in control subjects and patients with microalbuminuria, whereas control subjects and patients with microalbuminuria had nearly the similar medullary R2* value. This finding indicated that medullary hypoxia occurs in the earlier stage (normoalbuminuria stage), and then the medulla's oxygen consumption decreases to a level close to normal in the clinically early DN stage (microalbuminuria stage). The increased oxygen consumption in the renal medulla is thought to be mainly related to an increase in metabolic activity, such as glomerular hyperfiltration, active reabsorption of excess sodium, increased Na⁺/K⁺-ATPase activity.^{23–28} Dos Santos et al¹⁷ demonstrated that hypoxic changes can be detected by BOLD MRI as early as 2 days in diabetic rat kidneys. However, a study from Yin et al²² demonstrated that the medullary R2* values were evidently increased in simple diabetes and

the early stage of DN (microalbuminuria stage), whereas no significant difference was found between them, which is different from our result. In clinical practice, the appearance of microalbuminuria is usually used as the earliest clinical indicator of DN. But, this paradigm of early DN has been further questioned because a decrease in the renal function of a patient with diabetes is not always accompanied by increased albuminuria.³ There is a long silent period without overt clinical signs and symptoms of nephropathy prior to the onset of microalbuminuria. Accordingly, we assume that the renal progressive pathological changes have developed over a long silent period, but this subset of diabetic patients do not exhibit the proteinuria.²⁹ Based on this assumption, the medullary R2* value might be a new, more sensitive predictor of early DN, which could contribute to identifying these patients and intervening at an earlier time. Also, Palm et al²⁴ suggested such chronic oxygen deprivation, especially in the renal medulla,

Figure 2. (A) Box plot of medulla and cortical ADC values in healthy volunteers and patients with diabetic nephropathy. (B) Box plot of medulla and cortical FA values in healthy volunteers and patients with diabetic nephropathy. (C) Box plot of medulla and cortical R2* values in healthy volunteers and patients with diabetic nephropathy. *** indicates *p* < 0.001 ** indicates *p* < 0.01. ADC, apparent diffusion coefficient; FA, fractional anisotropy.

Figure 3. Correlations between ACR and ADC (A), FA (B), $R2^*$ (C). Correlations between eGFR and ADC (D), FA (E), $R2^*$ (F). The $R2^*$ value of the medulla has a weak correlation with ACR, and the other parameters had no correlation with ACR or eGFR. ACR, albumin-creatinine ratio; eGFR, estimated glomerular filtration rate; ADC, apparent diffusion coefficient; FA, fractional anisotropy; $R2^*$, the rate of spin dephasing.

could contribute to the progression of DN. Further research is needed to validate it.

DTI is another novel fMRI technique reflecting the diffusion of water molecules in anisotropy. The kidney is suitable for DTI examination due to its own special structure and physiological characteristics. The renal tubules, collecting systems, and blood vessels are arranged radially by the renal perineal nephrons and, thus, directional diffusion is more sensitive than average diffusion in assessing renal function and microstructure changes. The current study showed a decreasing trend in FA value of the renal medulla from the control group to the NAU group and to the MAU group; significantly lower FA values in the renal medulla of the MAU group than those in the control group were observed. This is consistent with the study by Chen et al.³⁰ It has been found that the microstructural alterations in the radially aligned tubules and/or vessels of the renal medulla could contribute to a decrease in FA value.^{9,31} Hyperglycaemia, a high-protein diet, and the effect of the renin-angiotensin-aldosterone system contribute to the increased tubular flow, hyperfiltration, and relative widening of the diameter of blood vessels and tubules,^{32,33} which may be earlier than the glomerular changes in diabetes. Sigmund et al.⁹ suggested that anisotropy of

the medulla of the kidney was associated with motion along the tubule, which was intratubular flow, and transtubular or across-tubule motion. Hueper et al.³⁴ postulated that decreased medullary FA may be associated with pathological changes such as glomerulosclerosis, tubulointerstitial injury, and tubular damage. Also, animal experiments have shown that DTI can detect changes in the renal parenchyma during the early phase of DN.^{11,34,35} Although there was no statistical difference, the medullary FA value was decreased successively from the control group to the NAU group to the MAU group, which also corresponds adequately with a known previous investigation¹²: DTI may identify early kidney changes in diabetics, and they identified significantly lower mean medullary FA values among diabetics with relatively intact renal function (eGFR > 60 mL/min/1.73 m²) compared with healthy control subjects. Y.Y. Yan et al.³⁵ applied DTI in a rat model study using streptozotocin-induced diabetes and found that FA is significantly reduced in diabetic nephropathy. They thought that FA might serve a potential role in the detection and therapeutic monitoring of early diabetic nephropathy. However, our finding is different from that of Chen et al.,¹¹ who demonstrated an increasing trend in FA value of the renal medulla across the three groups. Possible reasons are as follows: (1) our study was grouped according to ACR, their study

chose 8 h overnight urinary albuminuria excretion rate as the basis for grouping. (2) The machines and *b*-values adopted by the two institutes are different. MR scanner main magnet field strength are GE 3.0 T and PHILIPS 1.5 T, respectively. The *b*-value adopted by the first affiliated hospital of Jinan University is 0, 600, and there are 16 diffusion directions. The study of chen et al¹¹ uses *b*-value of 0, 500, and the diffusion direction number is 6. (4) ROI approaches used (Chen et al sampled FA maps in DTI and whole kidney tissue in ADC measurements) is different. (5) The inclusion criteria for the two studies were different.

In the current study, both BOLD imaging and DTI were employed to examine the kidneys in patients with diabetes. BOLD imaging detected the medullary hypoxia at the simple diabetes stage, whereas DTI did not identify the medullary directional diffusion changes at this stage. Subsequently, DTI exhibited the significant medullary directional diffusion abnormalities at the diabetic stage with microalbuminuria, whereas the medullary oxygen consumption regressed to the close to normal level at this stage. Based on our assumption mentioned previously, it is presumable that BOLD imaging could be more sensitive than DTI for assessment of the early renal function changes. Obviously, this presumption is a little arbitrary because of lack of pathohistological support for BOLD imaging and DTI parameter changes in our study. To be sure, BOLD imaging depicts the tissue oxygenation related to the tubular workload of the kidney. Apparently, FA value may not sensitive to the current energy metabolism or the tubular flow or reabsorption, but reflects the histopathological changes within the renal medulla.

Our study did not find that eGFR or ACR is associated with any value of DTI or BOLD except medullary R2*, which showed slightly negative correlation with ACR. In this experiment of ours, we may think that DTI and BOLD can more sensitively discover the early microstructural changes in DN. The specific mechanism needs to be tested in the future to confirm and improve. Similarly, Ries et al²³ found that oxygenation did not correlate with glycaemia or creatinine levels. The R2* value of the medulla in the control group has a weak correlation with ACR. However, there was no correlation between the ACR of diabetic patients and the renal cortex medulla DTI and BOLD values.

LIMITATIONS

The present study has the following limitations. A major limitation was the small sample size. Large numbers of patients with

diabetes may provide more precise values considering the additional changes in BOLD and DTI parameters. Another limitation was the limited subgroups of diabetes. We collected only the two subgroups of patients with diabetes (simple diabetes and diabetes with microalbuminuria), but diabetic patients in Stage IV and Stage V of DN were not included according with the Mogensen DN diagnostic criteria. As patients in Stage IV and Stage V of DN are clinically easy to diagnose, and there is generally no confusion. More subgroups may provide additional information about BOLD and DTI parameters changes with the progression of DN. Lack of direct histopathological evidence for renal BOLD and DTI parameters changes was the third limitation. Future longitudinal studies will need to be conducted to track these changes over time and get a further understanding of DN progression.

CONCLUSION

We employed a combination of BOLD imaging and DTI to detect the pathophysiological changes in patients with or without microalbuminuria. Our results showed that the hypoxia preceded diffusion anisotropy changes in the renal medulla in diabetic patients with normoalbuminuria. It suggests that BOLD imaging may be a earlier indicator modality than DTI for the non-invasive assessment of early renal structural/functional changes in patients with diabetes. Moreover, the change was mainly concentrated in the renal medulla. Does this indicate the treatment of early diabetic nephropathy should focus on improving the renal medulla oxygen metabolism was unknown? Those may provided a direction for further research on early diabetic nephropathy in the future.

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DISCLOSURE

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