ORIGINAL RESEARCH

Correlation Between Functional Magnetic Resonance Imaging and Renal Tubular Injury Markers in Early Assessment Of Renal Tubular Injury in Type 2 Diabetes Mellitus

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ABSTRACT

Objective • Both functional magnetic resonance imaging and renal tubular injury markers have been proved to be able to detect early renal damage in normoalbuminuric diabetic patients. This study mainly explored the functional magnetic resonance imaging parameters and renal tubular injury markers in the early evaluation of type 2 diabetes.

Methods • A case observation study was established, and 62 patients with early-stage low-risk type 2 diabetes mellitus with normoalbuminuric (UACR<30 mg/g, eGFR≥60 ml/min/1.73 m^2) were included for analysis. Urine kidney damage was determined by ELISA. Kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) assessment of renal tubular injury, and use of intravoxel incoherent motion magnetic resonance Imaging (intravoxel incoherent motion, IVIM) and blood oxygen level dependent magnetic resonance imaging (blood oxygen level dependent, BOLD) to evaluate renal cortex, medulla blood perfusion, water molecule diffusion, oxygenation level and other functional information, using linear correlation to analyze the correlation between functional magnetic resonance imaging parameters and markers of renal tubular injury.

Results • The correlation analysis between IVIM parameters and renal tubular injury markers showed that KIM-1 was inversely correlated with the MD value of the renal medulla region parameter (r = -0.24, *P* = .03), and was closely related to the other IVIM cortex and medulla. There was no correlation between qualitative parameters (*P* > .05), and no correlation between NGAL and all parameters of IVIM (*P* > .05). The correlation analysis between BOLD parameters and renal tubular injury markers showed that KIM-1 was positively correlated with renal medulla region parameter MR2* value (r = 0.26, *P* = .04) and MCR value (r = 0.28, *P* = .03), respectively. There was also a positive correlation between NGAL and renal medulla region parameter MR2* value (r = 0.24, *P* = .04).

Conclusion • In the early low-risk type 2 diabetic patients with normoalbuminuria, the more obvious the renal medullary water molecule diffusion disorder, the higher the renal tubular injury marker KIM-1, and the more severe renal medullary hypoxia, the renal tubular injury. The higher the markers KIM-1 and NGAL are, it is proved that the hypoxia and water diffusion disorder in the early renal medulla are related to renal tubular damage. (*Altern Ther Health Med.* 2024;30(5):235-243)

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INTRODUCTION

Although microalbuminuria and eGFR are still the most widely used clinical methods for early diagnosis of diabetic kidney disease (DKD),[1] their value in early diagnosis of DKD is limited, and better methods are needed for early detection of renal damage in DKD.

The "tubule-centric theory" suggests that renal tubules may replace the previously thought that glomeruli play a major role in the occurrence and development of early DKD, and renal tubular injury may be the "driving force" for the occurrence and development of DKD, which is earlier than eGFR decline and trace amount. Albuminuria should appear.[2,3]

Magnetic resonsnce imaging analysis can be used for evaluating the presence and sererity of early renal injuries.[4] DKD and diabetic peripheral neuropathy (DPN) are both

commom chronic complications of type 2 daibetes mellitus.[5] Many studies have shown that renal tubular injury markers can reveal early DKD tubular injury earlier than microalbuminuria,[6] and many of our previous studies have been suggested that functional magnetic resonance imaging can be combined with renal anatomy through non-invasive means, revealing the changes of renal function information in patients with early DKD without albuminuria, and indirectly reflecting the information of renal tubule-interstitial function by distinguishing and detecting the microstructural function information of the renal medulla, thereby early detection of renal tubular damage in diabetes.[7,8]

However, the correlation between renal tubular injury markers and functional magnetic resonance imaging in the evaluation of early DKD renal tubular injury is unclear. The tubular injury markers, such as kidney injury molecule 1, monocyte chemoattractant protein1 provide new opportunities to monitor response to therapeutics used to treat kidney disease, in addition, these markers can associated with the MR parameters, and these association can improve diagnositic accuracy and earlier assessment of therapeutic efficacy.Therefore, this study intends to include early low-risk type 2 diabetes mellitus with normoalbuminuria based on urinary microalbumin and eGFR. In patients (UACR < 30 mg/g, eGFR ≥ 60 ml/min/1.73 m^2), renal tubular injury markers (KIM-1, NGAL) were detected, and functional magnetic resonance imaging (IVIM, BOLD) was used to comprehensively evaluate renal skin, medulla blood perfusion, water molecule diffusion, oxygenation level and other renal function information, it may be the first time to explore the correlation between functional magnetic resonance imaging parameters and renal tubular injury markers, so as to further verify functional magnetic resonance imaging. The value of renal tubular injury markers in early evaluation of renal tubular injury in DKD.

OBJECTS AND METHODS

This study is a case-observation study that strictly follows the principles of the Declaration of Helsinki and the norms of clinical trials. All subjects were required to sign a written informed consent before the start of the study, and the study protocol was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Jinan University.

Research objects

Inclusion criteria. Type 2 diabetes patients who received routine treatment in the outpatient or inpatient department of the First Affiliated Hospital of Jinan University were screened, and the inclusion criteria were as follows: (1) In line with the "1999 WHO Diagnosis Criteria for Diabetes9"; (2) Aged more than 18 years old and less than 70 years old; (3) No other history of primary kidney disease other than diabetes and history of disease that may affect kidney function, such as: essential hypertension, gout, urinary tract infection, glomerulonephritis, nephrotic syndrome, kidney cancer, kidney stones wait; (4) Have not used diuretics recently, and have no history of taking drugs that obviously damage the kidneys; (5) Those who have no contraindications to magnetic resonance examination can successfully complete the magnetic resonance examination. (6) Obtain the informed consent and cooperation of the patients and their families, and sign the informed consent.

Exclusion criteria. (1) Proteinuria due to other primary or secondary causes, such as hypertensive emergency, high-protein diet, strenuous exercise, urinary tract infection, etc. (2) Combined with primary and other secondary kidney diseases, such as hypertensive nephropathy, glomerulonephritis, kidney stones, kidney transplantation, etc. (3) Acute complications such as severe infection, ketoacidosis, and hyperosmolar hyperglycemia. (4) Patients with severe organic lesions of the heart, lung, liver, brain and other organs. (5) MRI scan found that the renal structure was abnormal and affected the value of functional magnetic resonance imaging. (6) Pregnant and lactating women. (7) Type 1 diabetes and other special types of diabetes. (8) Significantly elevated blood glucose (fasting blood glucose ≥ 11.1 mmol/L) accompanied by obvious clinical symptoms of hyperglycemia. (9) Severe hyperlipidemia (low-density lipoprotein cholesterol ≥4.9 mmol/L or total high cholesterol ≥7.2 mmol/L or triglyceride ≥5.6 mmol/L) or severe hyperuricemia (serum uric acid ≥540 μmol /L).

Nephropathy criteria for inclusion. The patients were evaluated according to the recommendation of kidney disease improving global outcomes (KDIGO) for CKD risk stratification based on eGFR and albuminuria in 2012.[10] In patients with type 2 diabetes at risk, the rate of renal disease progression is slow, and the clinical characteristics include: (1) eGFR ≥ 60 ml/min/1.73 m2; (2) urinary ACR < 30 mg/g; (3) normal blood pressure, no Secondary renal damage such as primary hypertension and gouty nephropathy; (4) Routine urine examination is normal; (5) No renal structural abnormalities seen by imaging; (6) Being treated with conventional hypoglycemic regimens , fingertip blood sugar was well controlled; (7) ARB, ACEI, SGLT2 inhibitor and other treatment measures for diabetic nephropathy were not used. (8) Drugs that affect renal metabolism such as alprostadil and diuretics are not used.

Research methods

Record the basic information of the patient. Age, gender, height, weight, body mass index (BMI), blood pressure, duration of diabetes, past medical history, smoking history, family history, etc.

Testing routine laboratory indicators. (1) Detection of routine laboratory indicators (completed within three days before magnetic resonance examination): (i) Determination of fasting blood glucose, blood lipid profile, blood uric acid, liver function, blood creatinine, blood urea nitrogen and 2-hour postprandial blood glucose (full Automatic biochemical analyzer, Model 7600 Series, Hitachi); (ii) Using fasting fresh morning urine to measure urine microalbumin (automatic luminescence system, MAGLUMI 4000, Shenzhen

New Industry Biomedical Engineering Co., Ltd.) and urine creatinine (automatic biochemical Analyzer, Model 7600 Series, Hitachi), to calculate urinary albumin/creatinine ratio (UACR); (iii) Determination of glycated hemoglobin (HbA_{1c}) by high performance liquid chromatography (D-10 kit, Bio-Rad, USA). (2) Calculate eGFR according to the Chinese modified diet in renal disease (MDRD) formula.[11]

Experimental methods for the detection of renal tubular injury markers.

Urine specimen collection and storage. Collect 10 ml of fresh urine samples in the morning on an empty stomach, immediately centrifuge at 3000 rpm for 10 minutes at 4°C, and then use EP tubes to aliquot the urine supernatant and store in -80°C refrigerator until analysis, frozen samples Experiments should be carried out immediately after delivery to avoid repeated freezing and thawing.

Detection principle. The detection of KIM-1 and NGAL was based on enzyme-linked immunosorbent assay (ELISA), which was carried out by quantitative sandwich enzyme-linked immunosorbent assay technology. Monoclonal antibodies that specifically recognize KIM-1 or NGAL have been pre-coated on microplates. Add standards and samples to each well, in which KIM-1 or NGAL present binds to the immobilized antibody, and after washing away unbound material, an enzyme-linked monoclonal that specifically recognizes KIM-1 or NGAL is added to each well or polyclonal antibodies. After washing away the unbound enzyme-linked antibody reagent, add substrate solution to each well and develop color. The degree of color development is proportional to the amount of KIM-1 or NGAL initially bound. After the color development is terminated, the color intensity is measured and read. The color OD value was converted into the sample concentration according to the fitted standard curve.

Detection reagents. (1) Urinary KIM-1 ELISA kit Human Urinary TIM-1/KIM-1/HAVCR Quantizing ELISA Kit, catalog number DKM100, American R&D company, the specific reagent information is shown in Table 1. (2) Human Lipocalin-2/NGAL Quantikine ELISA Kit was used for urinary NGAL, catalog number DLCN20, American R&D Company. The specific reagent information is shown in Table 2.

Equipment and Instruments. The main experimental instruments are shown in Table 3.

Detection methods and steps of KIM-1. (1) KIM-1 reagent preparation: Store unopened kits at 2-8°C and return to room temperature for testing. (i) Wash Buffer (washing solution): Dilute 10ml Wash buffer (25X) with distilled water to prepare 250ml working concentration of washing solution. (ii) Substrate Solution: 15 minutes before use, mix equal amounts of Color Reagents A and B, protect from light, and add 200 µL of the mixture to each well. (iii) Calibrator Diluent RD6Q (diluent): Dilute 1:2, dilute 10ml of Calibrator Diluent RD6Q with distilled water to make a 20ml working concentration diluent. (iv) KIM-1 standard: dissolve 1 tube of standard with 1ml deionized water, the concentration is 100ng/ml, and let stand for 10 minutes. Take seven 1.5ml centrifuge tubes, label them A-G, add 900 µL of RD6Q to tube A, and add 200 µL to the remaining tubes. Take 100 µL from the dissolved standard and add it to tube A. After mixing, the concentration is 10ng/ml. Take 200 µL from tube A and add it to tube B. Do the same in sequence, and dilute 5 tubes of C-G. The dilution is used as blank control. (i.e., 0 ng/ml).

(2) KIM-1 experimental detection steps: Prepare all required reagents and standards as described in reagent preparation, and carry out experimental operations in strict accordance with the instructions. (i) Take out the microplate from the sealed aluminum foil bag that has returned to room temperature, put the unused strips back into the aluminum foil bag, and re-seal. (ii) Add 100 µL of Assay Diluent RD1-82 to all wells. (iii) Add 50 µL of standard curve and sample to each corresponding well, seal the reaction wells with sealing tape, shake for 10 minutes on a shaker at room temperature at 400 rpm, and incubate for 110 minutes. Record the intraplate location of the standards and experimental samples. (iv) After incubation, suck off the liquid in the plate, use a multi-channel pipette to add 300 µL of washing solution to each well, then shake off the washing solution in the plate, and tap on the absorbent paper to remove the residual liquid. Repeat the washing operation 3 times for a total of 4 washes. Aspirate the remaining liquid as much as possible each time the plate is washed. At the end of the last plate wash, blot all liquid from the plate, turn the plate upside down for a while, and then pat dry any residual liquid on absorbent paper. (v) Add 200 µL of Human TIM-1 Conjugate to each well, seal the reaction wells with sealing tape, shake for 10 minutes on a

Table 1. KIM-1 reagent information

Name	Quantity and Specification	Remarks
ELISA plate	1 plate	Use directly
Human TIM-1 Conjugate	21.5ml	Use directly
Human TIM-1 Standard	2vials	Preparation after dissolution
Assay Diluent RD1-82	11ml	Use directly
Calibrator Diluent RD6Q	21ml	Use after dilution
25X Wash Buffer	10ml	Use after dilution
Color Reagent A	12ml	Use directly
Color Reagent B	12ml	Use directly
Stop solution	6ml	Use directly

Table 2. NGAL reagent information

Name	Quantity and Specification	Remarks
ELISA plate	1 plate	Use directly
Human Lipocalin-2 Conjugate	21.5ml	Use directly
Human Lipocalin-2 Standard	2vials	Preparation after dissolution
Assay Diluent RD1-52	11ml	Use directly
Calibrator Diluent RD5-24	21ml	Use after dilution
25X Wash Buffer	10ml	Use after dilution
Color Reagent A	12ml	Use directly
Color Reagent B	12ml	Use directly
Stop solution	6ml	Use directly

Table 3. Experimental Instrument Information

Name	Brand/Company	Model
Microplate reader	BIOTEK	ELx808
water purifier	ASTK	CSR-1-10
Microplate Shaker	Chirim Bell	QB-9001
pipette	Miragen	-
small centrifuge	SciLogex	D1008
-80°C low temperature storage box	Thermo Fisher Scientific	907
-80°C low temperature storage box	Qingdao Haier Electric Co., Ltd.	DW-86W100
4±1°C medical refrigerator	Anhui Zhongke Duling Electric Co., Ltd.	MBC-4V 208
multifunctional centrifuge	Eppendorf	5811FN679213

shaker at 400 rpm at room temperature, and incubate for 110 minutes. (vi) Repeat the plate washing operation in step (iv), and wash the plate 4 times. (vii) Add 200 μL of Substrate Solution to each well, shake for 5 minutes on a shaker at 400 rpm at room temperature, and incubate for 25 minutes. After adding the chromogenic solution, cover it with a blackboard cover to protect it from light. During the incubation period, the OD value at 630 nm wavelength can be detected to prevent the OD value from being too high and exceeding the detection limit of the microplate reader. (viii) Add 50 μL of stop solution to each well, the color of the solution in the well will change from blue to yellow, if the color of the solution changes to green or the color changes are inconsistent, gently shake the microplate to mix the solution evenly. Within 30 minutes after adding the stop solution, use a microplate reader to measure the absorbance value at 450 nm, and set 540 nm as the calibration wavelength. (ix) According to the concentration of the standard product and the corresponding absorbance value, take the concentration of the standard product as the abscissa, and OD450-540 as the ordinate (if multiple wells are set, the average value should be taken), using the software sigmaplot to select four-parameter fitting The function is used to draw a standard curve, and the concentration of KIM-1 in the sample is calculated from the standard curve through the sample OD value. (x) The final sample concentration level needs to be corrected by urine creatinine, expressed by dividing by the ratio of urine creatinine, and the subsequent analysis of the results is carried out.

NGAL detection methods and steps. (1) NGAL reagent preparation: The Human Lipocalin-2 Conjugate in the kit needs to be kept at 2-8°C, and other reagents should be warmed to room temperature before detection. (i) Wash Buffer (washing solution): Dilute 10ml Wash buffer (25X) with distilled water to prepare 250ml working concentration of washing solution. (ii) Substrate Solution: 15 minutes before use, mix equal amounts of Color Reagents A and B, protect from light, and add 200 μL of the mixture to each well. (iii) Calibrator Diluent RD5-24 (diluent): Dilute at 1:5, dilute 20ml of Calibrator Diluent RD5-24 with distilled water to make a 100ml working concentration of diluent. (iv) NGAL standard: dissolve 1 tube of standard with 1ml deionized water, the concentration is 100ng/ml, and let stand for 10 minutes. Take seven 1.5ml centrifuge tubes, mark them A-G, add 900 μL of RD5-24 to tube A, and add 200 μL to the remaining tubes. Take 100 μL from the dissolved standard and add it to tube A. After mixing, the concentration is 10ng/ml. Take 200 μL from tube A and add it to tube B. Do the same in sequence, and dilute 5 tubes of C-G. The dilution is used as a blank control (i.e., 0ng/ml). (v) Sample to be tested: Dilute the sample 10 times with RD5-24 before loading the sample. The diluted sample is first placed at 4°C, and after the standard is prepared, it is added to the ELISA plate for incubation.

(2) NGAL experimental detection steps: Prepare all required reagents, standards and samples as described in reagent preparation, and carry out experimental operations in strict accordance with the instructions. (i) Take out the microplate from the sealed aluminum foil bag that has returned to room temperature, put the unused strips back into the aluminum foil bag, and re-seal. (ii) Add 100μL of Assay Diluent RD1-52 to each well. (iii) Add 50 μL of standard curve and sample to each corresponding well, seal the reaction well with sealing tape, shake for 10 minutes on a shaker at 400 rpm at room temperature, and then transfer to a 4°C refrigerator to incubate for 110 minutes. Record the intraplate location of the standards and experimental samples. (iv) After incubation, suck off the liquid in the plate, use a multi-channel pipette to add 300 μL of washing working solution to each well, then shake off the washing solution in the plate, and tap on absorbent paper to remove the residual liquid. Repeat the washing operation 3 times for a total of 4 washes. Aspirate the remaining liquid as much as possible each time the plate is washed. At the end of the last plate wash, blot all liquid from the plate, turn the plate upside down for a while, and then pat dry any residual liquid on absorbent paper. (v) Take out the Human NGAL Conjugate from the refrigerator, add 200 μL to each well, seal the reaction wells with sealing tape, shake on a shaker at room temperature at 400 rpm for 10 minutes, and transfer to a 4°C refrigerator to incubate for 110 minutes. (vi) Repeat the plate washing operation in step (iv), and wash the plate 4 times. (vii) Add 200 μL of Substrate Solution to each well, shake for 5 minutes on a shaker at 400 rpm at room temperature, and incubate for 25 minutes. After adding the chromogenic solution, cover it with a blackboard cover to protect it from light. During the incubation period, the OD value at 630nm wavelength can be detected to prevent the OD value from being too high and exceeding the detection limit of the microplate reader. (viii) Add 50 μL of stop solution to each well, the color of the solution in the well will change from blue to yellow, if the color of the solution changes to green or the color changes are inconsistent, shake the microplate gently to mix the solution evenly. Within 30 minutes after adding the stop solution, use a microplate reader to measure the absorbance value at 450 nm, and set 540 nm as the calibration wavelength. (ix) According to the concentration of the standard product and the corresponding absorbance value, take the concentration of the standard product as the abscissa and OD450-540 as the ordinate (if multiple wells are set, the average value should be taken), and use the software sigmaplot to select four-parameter fitting The standard curve is drawn by the function, and the detection concentration of NGAL in the sample is calculated from the standard curve through the OD value of the sample, and the final detection concentration is multiplied by 10 to obtain the actual sample concentration. (x) The actual sample concentration level needs to be corrected by urine creatinine, which is expressed by dividing by the ratio of urine creatinine and the subsequent analysis of the results.

Functional Magnetic Resonance Imaging.

Magnetic resonance examination equipment and preparation before scanning. The magnetic resonance imaging equipment used a 3.0T magnetic resonance imaging

system (Discovery MR750, GE Healthcare, WI, USA) for scanning measurement, an 8-channel body coil was used for scanning, and a post-processing workstation (Sun Advantage Workstation 4.5, SDW4.5) was used for scanning Image post-processing and parameter acquisition and analysis. Before the MRI scan, all subjects should fast for more than 2 hours, drink no more than 350 ml of water within 2 hours, stop taking any drugs within 2 hours before the examination, train end-expiratory breath-holding before the examination, and the subjects are in a supine position. Foot advanced, coronal scan, and breathing straps on the mid-abdomen.

Magnetic resonance scan sequence and parameter setting. (1) Conventional magnetic resonance imaging sequence: (i) Localization image scan: 3-Planes FSEloc sequence (SSFSE), repetition time: 1141ms, echo time: 77.8ms, slice thickness: 8.0mm, interval: 0.0mm, field of view: 44cm× 44cm, matrix: 384×l60, excitation times: 0.55, scan time: 20s. (ii) Coronal T1WI: FSPGR sequence, repetition time: 3.7ms, echo time: 1.1ms, slice thickness: 6.0mm, slice interval: 3.0mm, field of view: 40cm×40cm, bandwidth: 166.7kHz, matrix: 256×200, Number of excitations: 0.69, scan time: 5s. (iii) Coronal (SSFSE) BH-T2WI: repetition time: 1875ms, echo time: 67.0ms, slice thickness: 5.0mm, slice interval: 1.0mm, field of view: 40cm×40cm, bandwidth: 83.3kHz, matrix: 288×288, excitation times: 0.53, scan time: 21s. (iv) Coronal (FRFSE/Prop) RTr-T2WI: repetition time: 6000ms, echo time: 73.9ms, slice thickness: 5.0mm, slice interval: 1.0mm, field of view: 40cm×40cm, bandwidth: 83.3kHz, matrix: 320 ×320, excitation times: 2.5, scan time: 108s.

(2) Coronal IVIM imaging sequence: using SE/EPI sequence, repetition time: 6667ms, echo time: 51.8ms, slice thickness: 5.0mm, slice interval: 1.0mm, field of view: 40.0cm×32cm, bandwidth: 250kHz, Matrix: 128×160, number of excitations: 4.0, acquisition time: 267s. 10 to 12 slices of coronal images were obtained for each kidney, and 11 frames of images with different b values (0, 30, 50, 80, 100, 150, 200, 300, 500, 800, 1000s/mm^2) were obtained for each slice.

(3) Coronal BOLD imaging sequence: using a modified multi-echo data image combination sequence (MEDIC), the specific parameters are as follows: 8 echoes, repetition time: 150 ms, echo time: 3.4-27.8 ms, slice thickness: 5.0 mm, slice interval: 1.0 mm, flip angle 30°, field of view: 40.0 cm×32 cm, bandwidth: 50 kHz, matrix: 256 × 192, number of excitations: 1.0, scan time: 45-52s.

Magnetic resonance image processing and parameter value measurement. The renal morphological evaluation of all patients was performed on coronal T2WI. Two physicians with more than 5 years of experience in abdominal imaging diagnosis independently completed image analysis and data collection. If there is a disagreement between the two, it will be decided after discussion. Secondly, the two completed image analysis and parameter value measurement independently, and neither of them knew the clinical information of the patient before the measurement.

The original image data of the two imaging methods, IVIM and BOLD, were respectively transferred to the post-processing workstation (Sun Advantage Workstation 4.5, ADW 4.5) for image post-processing, and the parametric images of each imaging sequence were obtained. Diaphragmatic motion artifacts have less impact than the left kidney, so the right kidney was selected for parameter value measurement. At the coronal plane near the renal hilum, the renal blood vessels, dilated renal pelvis, renal calyces and renal margins were avoided as far as possible, and the renal skin and spinal cord were placed on the kidney skin and marrow. Three regions of interest (ROI) were selected for parameter value measurement at the upper, middle and lower poles. The size of the ROI of IVIM was 45-60mm^2 (medulla was 45-55mm^2, and cortex was 50-60mm^2), BOLD The size of the ROI ranged from 45-60 mm^2 (medulla was 45-55 mm2, cortex was 50-60 mm2), and the parameter values of each imaging method were taken as the average of the respective ROI measurements, and the overall measurement process should be repeated three times independently, take the average of three measurement values for later statistical analysis, and the specific parameter values include IVIM parameter values (ADC value, D value, D* value, f value) and BOLD parameter value (R2* value). The ROI selection method and specific parameter collection process of IVIM and BOLD refer to the previous research of our group.[7,8]

Statistical analysis

Data for continuous variables are represented by mean ± standard deviation if they conform to a normal distribution, median (interquartile range) if skewed, and frequencies (percentages) for categorical data. The correlation between variables was analyzed by Pearson correlation analysis (data that conformed to normal distribution) or Spearman correlation analysis (data that did not conform to normal distribution). All statistical analyses were performed using SPSS 19.0 software and were considered statistically significant if statistically $P < .05$.

Technical Process

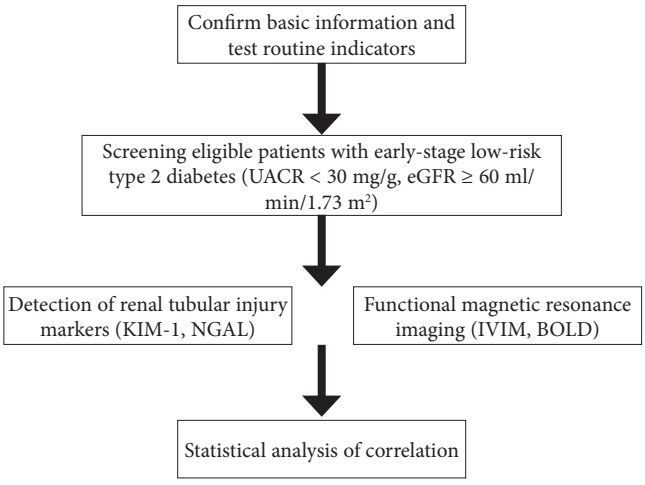


Table 4. Basic clinical data of the included patients

Clinical information	Diabetic patients (n = 62)
Gender (Male/Female)	30/32
age	56.77±8.13
Duration of diabetes (years)	6.78(3.0-10.0)
Smoking history n (%)	12(19.35)
BMI (kg/m^2)	23.62±2.54
HbA1c (%)	7.23(5.62-8.08)
Systolic blood pressure (mmhg)	124.84±12.41
Diastolic blood pressure (mmhg)	77.13±10.01
Fasting blood glucose (mmol/L)	6.54±1.42
2-hour postprandial blood glucose (mmol/L)	8.86±1.18
Serum creatinine (μmol/L)	68.05±16.74
Serum uric acid (μmol/L)	351.36±91.57
Total cholesterol (mmol/L)	4.83±1.19
Triglycerides (mmol/L)	1.66±1.19
Low density lipoprotein (mmol/L)	2.71±0.86
High-density lipoprotein (mmol/L)	1.04±0.26
UACR (mg/g)	10.50±7.11
eGFR(ml/min/1.73m^2)	94.43±14.86

Table 5. Detection data of functional magnetic resonance parameters and renal tubular injury markers

Related Indicators	Detection value (n = 62)
CADC (10^{-3}mm^2/s)	1.93±0.48
CD (10^{-3}mm^2/s)	1.69±0.22
CD* (10^{-3}mm^2/s)	15.36±8.55
Cf (%)	28.17±6.85
CR2*(ms^{-1})	17.24±2.88
MADC (10^{-3}mm^2/s)	1.93±0.22
MD (10^{-3}mm^2/s)	1.63±0.28
MD*(10^{-3}mm^2/s)	15.52±7.94
Mf (%)	20.16±8.61
MR2*(ms^{-1})	26.17±3.3
MCR	1.61±0.22
UKIM-1/CR(μg/g)	1.18±0.66
UNGAL/CR(μg/g)	23.33±4.68

Abbreviations: CADC, cortical ADC value; CD, cortical D value; CD*, cortical D* value; Cf, cortical f value; CR2*, cortical R2* value; MADC, medulla ADC value; MD, medulla D value; MD*, medulla D* value; Mf, medulla f value; MR2*, medulla R2* value; MCR, ratio of MR2* to CR2*; UKIM-1/CR, ratio of urinary KIM-1 to urinary creatinine; UNGAL /CR, urinary NGAL to urinary creatinine ratio.

Table 6. Correlation analysis between functional magnetic resonance parameters and renal tubular injury markers

	UKIM-1/CR		UNGAL/CR	
	r	*P* value	*r*	*P* value
CADC	0.12	0.31	0.03	0.76
CD	-0.11	0.32	-0.18	0.11
CD*	0.07	0.57	0.08	0.45
Cf	0.01	0.96	0.05	0.65
CR2*	0.16	0.21	0.04	0.72
MADC	0.22	0.08	0.05	0.68
MD	-0.24	0.03	-0.04	0.69
MD*	0.08	0.48	0.03	0.77
Mf	0.18	0.12	0.11	0.31
MR2*	0.26	0.04	0.24	0.04
MCR	0.28	0.03	0.09	0.49

Abbreviations: CADC, cortical ADC value; CD, cortical D value; CD*, cortical D* value; Cf, cortical f value; CR2*, cortical R2* value; MADC, medulla ADC value; MD, medulla D value; MD*, medulla D* value; Mf, medulla f value; MR2*, medulla R2* value; MCR, ratio of MR2* to CR2*; UKIM-1/CR, ratio of urinary KIM-1 to urinary creatinine; UNGAL /CR, urinary NGAL to urinary creatinine ratio.

RESULTS

Basic clinical information

According to the inclusion and exclusion criteria, patients who were affected by the analysis due to magnetic resonance artifacts and hidden structural lesions of the kidney were excluded, and 62 patients were finally included for the final analysis. Table 4 summarizes the basic clinical data of the included patients.

Functional magnetic resonance parameters and detection results of renal tubular injury markers

The experimental detection of renal tubular injury markers (including KIM-1 and NGAL) was strictly carried out in accordance with the steps in the kit instructions. All parameters of the standard curves of the two met the kit quality control requirements, indicating that the kit and experimental operations were in line with the requirements of the kit. requirements, the results are reliable. The concentration results of both samples were corrected by urine creatinine, and both were divided by urine creatinine to obtain their respective ratios to urine creatinine, which were expressed as UKIM-1/CR and UNGAL/CR, respectively, and the subsequent results were analyzed by this ratio.

All included patients had good fMRI imaging and smooth parameter acquisition. The consistency test of IVIM and BOLD imaging parameter values detected by two diagnostic imaging physicians respectively showed that the correlation coefficients were all greater than 0.8, indicating that the values of all parameters were consistent and the results were reliable.

Table 5 summarizes the values of IVIM imaging parameters (CADC, CD, CD*, Cf, MADC, MD, MD*, Mf), BOLD imaging parameters (CR2*, MR2*, MCR) and markers of tubular injury for the population of included patients The detection value of substances (UKIM-1/CR, UNGAL/CR)

Correlation analysis between functional magnetic resonance parameters and renal tubular injury markers

The correlation analysis of IVIM parameters with UKIM-1/CR and UNGAL/CR indicated that UKIM-1/CR was negatively correlated with the renal medulla region parameter MD value (r = -0.24, *P* = .03) (see Table 6, Figure 1), and other IVIM parameters (CADC, CD, CD*, Cf, MADC, MD*, Mf) had no correlation (*P* > .05). However, there was no correlation between UNGAL/CR and all parameters of IVIM skin and medulla imaging (CADC, CD, CD*, Cf, MADC, MD, MD*, Mf) (*P* > .05).

The correlation analysis of BOLD parameters with UKIM-1/CR and UNGAL/CR showed that UKIM-1/CR had no correlation with renal cortical parameter CR2* value (p>0.05), but UKIM-1/CR was associated with renal cortical parameters respectively. There was a positive correlation between the medulla region parameter MR2* value (r = 0.26, *P* = .04) and MCR value (r = 0.28, *P* = .03) (see Table 6, Figure 2, Figure 3). UNGAL/CR was positively correlated with the renal medulla region parameter MR2* value (r = 0.24, *P* = .04) (see Table 6, Figure 4), but had no correlation with CR2* and MCR values (*P* > .05).

DISCUSSION

The commonly used clinical eGFR and microalbuminuria have obvious limitations in the early diagnosis of DKD. People still need to explore and verify better technical means to detect DKD early. The "tubule-centric theory" suggests that renal tubules may replace the previously thought that

Figure 1. Scatter plot of correlation between UKIM-1/CR and renal medulla MD value

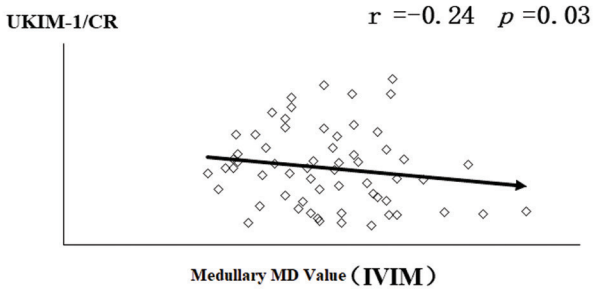


Figure 2. Scatter plot of correlation between UKIM-1/CR and renal medulla MR2* value

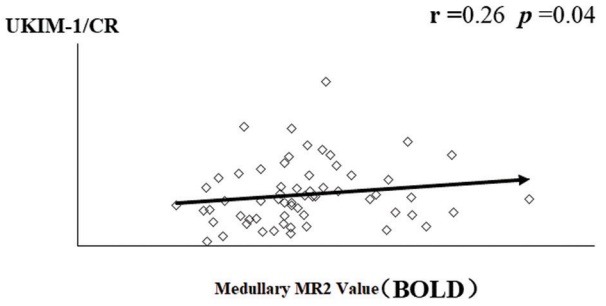


Figure 3. Scatter plot of correlation between UKIM-1/CR and MCR value

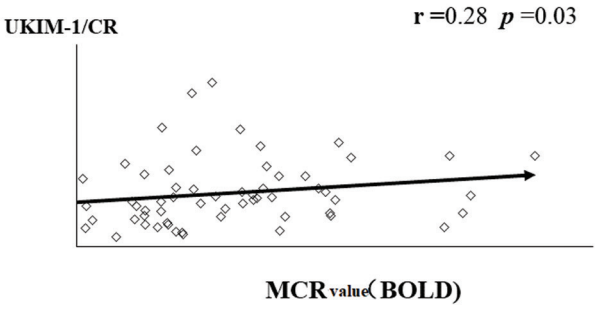
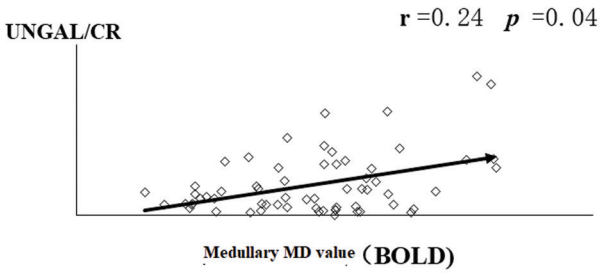


Figure 4. Scatter plot of the correlation between UNGAL/CR and renal medulla MR2* value



glomeruli play a leading role in the occurrence and development of early DKD, and renal tubular injury may be the "driving force" for the occurrence and development of DKD. Proteinuria should have appeared. Markers of renal tubular damage can reveal the renal tubular damage in early diabetes, and functional magnetic resonance imaging has also been proved to be able to combine renal anatomy, through non-invasive means, to reveal the changes of renal function information in early diabetes, by distinguishing and detecting renal medullary micro-organisms. Structural function information indirectly reflects the information of renal tubule-interstitial function, so as to detect renal tubular damage in diabetes early. Therefore, renal tubular damage markers and functional magnetic resonance imaging have certain application value in the evaluation of early DKD, and both may be useful. Reflecting the early renal tubular injury, there may be a certain degree of correlation.

Correlation between renal tubular injury markers and IVIM parameters

The main parameters of IVIM include: ADC: mainly reflects the diffusion movement of water molecules and blood perfusion-related diffusion; D: reflects the simple diffusion movement of water molecules, generally excluding blood perfusion-related diffusion; f: perfusion-related volume fraction, refers to the proportion of diffusion related to microcirculation perfusion in total diffusion reflects the abundance of microvessels; D*: diffusion coefficient related to capillary perfusion, reflecting the diffusion related to local tissue perfusion. A number of previous studies including our group have proved[7,12,13] that IVIM can detect information changes in kidney skin, medullary water molecule diffusion, and blood flow microperfusion in the early stage of diabetes. There may be some correlation between IVIM parameters and renal tubular injury markers.

This study found that there was no significant correlation between NGAL and renal cortex, medulla ADC, D, D*, and f parameters, and the difference was not statistically significant, while KIM-1 was associated with all parameters in cortical area and medulla area parameters MADC, MD*. There was no significant correlation between, Mf, but it was negatively correlated with the MD value of the renal medulla region, and the difference was statistically significant. Since both NGAL and KIM-1 are markers of renal tubular injury, and the renal cortex mainly contains glomerular structures, it may explain that there is no significant correlation between NGAL and KIM-1 and the IVIM parameters in the cortex. However, KIM-1 and NGAL were not significantly correlated with the medulla regional parameters MADC, MD*, Mf despite the presence of a large number of tubular structures in the medulla, which may be because ADC, D*, f are all related to perfusion.[14] The parameters of β-diabetes, previous studies have shown,[15] in the relatively high filtration state of diabetic patients (mean 135ml/min/1.73 m^2), renal tubular injury markers have been increased, and renal tubular injury markers (urine KIM- 1, NGAL, etc.) are positively correlated with eGFR, which may be due to the early high filtration state, which leads to the existence of high perfusion state in

medullary renal tubules, and renal tubules actively reabsorb too much glucose, consume too much energy, and make renal tubules in hypoxia Injury is a manifestation of early renal tubular function damage, however, another study by the same author showed that,[16], in patients with approximately normal eGFR (mean 85.9 ml/min/1.73 m^2). there is no significant correlation with renal tubular injury markers. Therefore, it can be considered that when the filtration rate is not significantly increased, there may be no significant correlation between perfusion and renal tubular injury. There was no significant correlation between NGAL, KIM-1 and perfusion-related parameters ADC, D*, f.

This study found that KIM-1 was negatively correlated with the MD value of the medulla region parameter, which indicated that the higher the KIM-1 value, the lower the medulla MD value. The D value reflects the simple diffusion movement of water molecules. Previous studies of our group have confirmed that the MD value of the renal medulla decreases in diabetic patients without early albuminuria, suggesting that the early water molecule diffusion disorder may be mainly related to early renal tubular damage,[7] the decrease of renal medulla MD value indirectly reflects the early renal tubular water diffusion dysfunction. Combined with the results of this study, we found that the more severe the renal tubular damage (the increase of KIM-1), the more serious the renal medullary renal tubular water diffusion barrier, and the markers of renal tubular damage can also reveal the early stage of microalbuminuria. Therefore, the correlation with each other may be more able to reflect the application value of each other in the early assessment of DKD renal tubular injury, and more able to prove the existence of renal tubular injury in the early stage of diabetes.

Correlation between renal tubular injury markers and BOLD parameters

Tubular hypoxia is an important pathophysiological mechanism of early renal tubular damage, which may be a characteristic of diabetes mellitus.[17] BOLD imaging technology is an ideal technical means to non-invasively evaluate the level of renal oxygenation, and can combine anatomical information to understand the oxygenation of the renal cortex and medulla, especially the oxygenation of the medulla, which has been proved to be able to evaluate early the main parameter of renal tubulointerstitial oxygenation in diabetic patients is R2*. The higher the R2* value, the more severe the hypoxia in the tissue. Therefore, there may be some correlation between BOLD parameters and renal tubular injury markers.[18,19]

This study found that there was no significant correlation between NGAL, KIM-1 and the cortical area parameter CR2* value. Previous studies of our group have shown[8] that in early diabetic patients without albuminuria, the cortical CR2* value did not increase significantly, and the cortical glomerular blood flow was abundant in early diabetic patients, and the cortical oxygenation was good. It is the small part of the renal tubular located in the cortex that the increased oxygen consumption is not enough to offset the effect of good renal perfusion, and the glomerular filtration rate in this study population is relatively early, so the oxygenation of the early renal cortex is relatively good. There was little relationship with markers of renal tubular injury.

However, this study found that NGAL and KIM-1 had a significant positive correlation with the MR2* value of the medulla region parameter. Previous studies of our group have shown[8] that the renal medulla MR2* of early diabetic patients without albuminuria is higher than that of normal healthy controls, which proves that early diabetic patients have renal medullary hypoxia, which may be due to renal medulla. The blood supply is significantly lower than that of the cortex, and the medullary blood flow is less than 10% of the renal blood flow, but the metabolic activity of the medullary tubules is almost entirely aerobic. In fact, 60% of the total energy consumption of the kidney is through renal tubular sodium. Caused by glucose reabsorption, which is an active energy consumption process, so the medulla is more sensitive to hypoxia. The hypoxic state of the renal medulla is mainly related to excessive glucose reabsorption by renal tubular active energy consumption, which is also type 2 diabetes. One of the mechanisms of early renal tubular injury, and at this early stage, many studies have also proved that the renal tubular injury markers NGAL and KIM-1 have also been increased, so this study found that the renal tubular injury marker and the medulla MR2* value are related. Obviously positive correlation, indicating that the more severe the renal medullary hypoxia, the more serious the renal tubular damage, to a certain extent, it may more verify the existence of early diabetic renal tubular hypoxia damage.

Many studies[20,21] suggest that the MCR value increases most significantly in patients with simple non-albuminuric diabetes mellitus, which is higher than that of normal healthy controls or middle and advanced DKD. The reason may be due to the rich blood supply of the cortex itself. The glomerular filtration rate decreased, and the cortex gradually showed hypoxic state, so the cortex CR2* gradually increased in the middle and late stages of DKD, while the hypoxia state of the medulla improved with the progress of DKD, and the medulla MR2* gradually decreased, which may be due to Decreased glomerular filtration rate reduces active tubular reabsorption and reduces oxygen consumption. This study found that KIM-1 has a significant positive correlation with MCR value, which may be due to the fact that the subjects included in this study were simple diabetic patients without albuminuria in the early stage, and the glomerular filtration rate did not decrease significantly at this stage. With the increase of the glomerular filtration rate, the cortical blood supply perfusion is better, and the cortical CR2* gradually decreases. However, the increase of perfusion will aggravate the excessive dynamic energy consumption and reabsorption of glucose in the medullary renal tubules, and excessive active energy consumption. Tubular energy load, medullary hypoxia aggravates, and medullary MR2* gradually increases. Therefore, in this early stage of low-risk diabetes, a higher

MCR value may represent more severe medullary hypoxia and more severe tubular hypoxia. The more severe the renal tubular damage, the study found that KIM-1 had a significant positive correlation with MCR value, which verifies this view to a certain extent.

Therefore, combined with the results of this study, we found that with the aggravation of renal medulla hypoxic injury, renal tubular injury markers increased, which to a certain extent may verify that renal tubular hypoxia is an important pathophysiology of early renal tubular damage. Mechanism, renal tubular hypoxic lesions exist in the early stage of diabetes, which promotes the occurrence and development of DKD.

Limitations

No renal histopathological examination was performed to confirm the evidence of renal pathological damage in this study. Moreover, the small number of patients, and the statistical analysis need to go deep, because there were too many factors that would affects the results, so in another study we also include Cox regression analysis to avoid the covariables which can affect the results.

CONCLUSIONS

In patients with early-stage low-risk type 2 diabetes mellitus with normoalbuminuria, the more obvious the renal medullary water molecule diffusion disorder, the higher the renal tubular injury marker KIM-1, and the more severe renal medullary hypoxia, the higher the renal tubular damage. The higher the damage markers KIM-1 and NGAL are, it is proved that the hypoxia and water diffusion disorder of the early renal medulla are related to the renal tubular damage.

DATA AVAILABILITY

The experimental data used to support the findings of this study are available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest regarding this work.

FUNDING STATEMENT

Medical Research Foundation of Guangdong Province Grant Number: B2023376; Scientific research project of Guangdong Provincial Bureau of traditional Chinese Medicine Grant Number: 20231083; Guangzhou Science and Technology Planning Project Grant Number: 2023A04J1909.

AUTHOR CONTRIBUTIONS

Chuangbiao Zhang and Beibei Zhu contributed equally to this work

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