

Original Article

Diffusion-weighted imaging using 3T MRI to evaluate mesangial proliferative glomerulonephritis: an experimental study in rabbits

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Abstract: Purpose: To investigate the feasibility of magnetic resonance imaging (MRI) Diffusion weighted imaging (DWI) in evaluating rabbit Mesangial Proliferative Glomerulonephritis (MsPGN). Methods: 16 rabbits with MsPGN and 8 normal rabbits (group A) were performed conventional MR and DWI. The rabbits with MsPGN were divided into 2 groups, 4 weeks (group B) and 8 weeks (group C) after model, respectively. Results: The cortical relative apparent diffusion coefficient (rADC) value in all three groups was higher than medullary. There were statistical differences between cortical rADC and medullary rADC value in group A, and so was in group B. rADC values of cortex and medulla of groups B and C decreased compare to those of group A. There were statistical differences between groups A and C in cortical rADC value, as well as between groups B and C. There were no significant differences among the three groups in medullary rADC value. The cortical fractional anisotropy (FA) value was lower than medullary in all three groups and there were statistical differences in groups A, B and C. Cortical FA values of groups B and C were gradually decreased compared to group A. There were statistical differences between cortical FA value between groups A and C, groups B and C, as well as groups A and B. There was no statistical difference in medullary FA value among the three groups. Conclusions: DWI has potential for diagnosis of MsPGN and assessing the severity of renal pathology, especially for cortical FA value, which is a non-invasive and effective technique for guiding therapy and follow-up in MsPGN patients.

Keywords: Mesangial proliferative glomerulonephritis, magnetic resonance imaging, diffusion-weighted imaging, animal model, rabbit

Introduction

Mesangial proliferative glomerulonephritis (MsPGN) is one of the important pathological types of primary glomerulonephritis (GN). The prevalence of MsPGN in primary glomerulonephritis is about 7%-32%, which is obtained from renal biopsy data. In China the average morbidity is 29.7%-36%, which could be as high as 57.4% for children [1]. Mesangial cell proliferation frequently precedes and relates to the increase of extracellular matrix in the mesangium of MsPGN, which has prominent accordance with clinical manifestation and prognosis. The earliest clinical evidence is hematuria, microalbuminuria and nephrotic syndrome which can progress to end-stage renal disease [2]. Renal biopsy is the most common tech-

nique to identify the type and severity of renal pathology and is vital in guiding treatment and assessing outcome and prognosis. However, renal biopsy also carries risks such as hematuria, perirenal hematoma, arteriovenous fistula, infection, and even death. Furthermore, biopsy is not an ideal repeatable non-invasive alternative for follow-up due to uncertain sampling errors and observer bias. Serum creatinine concentration is dependent on body mass and is a late marker for renal dysfunction without capacity of split renal function evaluation [3, 4].

Computer tomography (CT) and ultrasound (US) provide anatomical information of the kidneys. However, CT scan has radiation exposure and the use of iodinated contrast agents has the risk of allergies and kidney damage, whereas

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US is strongly user dependent. Functional information can also be derived from nuclear medicine examinations, but administration of radioactive tracers is required and spatial information is low. Diffusion weighted imaging (DWI) is a non-invasive magnetic resonance imaging (MRI) technology that reflects the apparent diffuse movement of water molecules and is quantitated by the apparent diffusion coefficient (ADC). Magnetic resonance diffusion weighted imaging may detect renal impairment and pathology in native and transplanted kidneys both in human and animal studies [3-5]. Previous studies show that ADC values decrease in chronic kidney disease and depend on the degree of impairment of renal function [6]. While diffusion weighted imaging measures the magnitude of water molecules diffusion, diffusion tensor imaging (DTI) provides additional information about diffusion direction and degree of directed diffusion (fractional anisotropy, FA). Renal DTI has recently been applied for detection of allograft dysfunction in humans and ischemia reperfusion injury in a rat model [7, 8]. Repeated measurements of the kidneys in healthy volunteers proved reproducible on DW MR imaging [9]. These results confirm the reliability of this method for the follow-up of different renal abnormalities. The purpose of this study was to investigate whether MR DWI and DTI are able to detect pathological changes of MsPGN and to determine the correlation of ADC and FA to the degree of renal pathologies in a rabbit model of MsPGN. The accuracy for ADC quantification may be influenced by the imaging parameters, such as field strength, b-values, and even the individual's physical state [10, 11]. Choosing a reference organ in ADC calculations may reduce variability across research centers, observers, and patients. We choose the erector spinae muscles in the same layer of kidney hilar for reference site. rADC, defined as the ratio of kidney ADC to erector spinae muscles.

Material and methods

Animals

All study methods were approved by ethics committee of Nanjing First Hospital, Nanjing Medical University (Nanjing, China). Six to eight week old healthy, male rabbits weighted 1.7 kg-2.3 kg were obtained from the Vital River

Laboratory Animal Co., Ltd., Beijing Laboratory Research Center (Beijing, China); and were housed in specific pathogen-free conditions at Nanjing First Hospital Animal Center (Nanjing, China). These rabbits have free access to standard diet and water.

Animal treatment

Animals were randomly divided into three groups including control group (8 rabbits) and 2 model groups (8 rabbits for 4 weeks group and 8 rabbits for 8 weeks group). Rabbits in model groups were modeled by injection of bovine serum albumin (BSA) (250 mg/kg) via auricular vein, which was successfully repeated by many researchers in China [2]. The control group only received an injection of same amount of saline underwent the same procedure. At 4 and 8 weeks after injection, control and model groups were examined by MRI. After MRI exam we executed 2 rabbit in each group to obtain samples of kidneys. Routine HE stain and Periodic acid-Schiff (PAS) staining were used to determine glomerular damage including mesangial cell proliferation and the increase of extracellular matrix in the mesangium.

MRI protocol

Four and eight weeks after BSA injection, rabbits underwent MRI (3.0 T Achieva TX Series, Philips, Holland) using an 8-channel knee coil. Anesthesia was induced by intra-peritoneal injection of 0.5 ml/kg of 8% chloral hydrate. All images were acquired with respiratory triggering technology. Respiratory motion was reduced by imaging the animals in the supine position. Spin echo images were acquired in T2WI (axial planes, TR/TE = 1487.6/100 ms; section thickness = 3 mm; number of sections = 24) and T1WI (oblique coronal planes, TR/TE = 500/20 ms; section thickness = 3 mm; number of sections = 18). The coronal plane was orientated along the long axis of the left kidney, and FOV and matrix were adjusted to obtain an isotropic in-plane resolution. For diffusion measurements, a fat-saturated (spectral presaturation inversion recovery, SPIR), single-shot spin echo planar DTI sequence was applied matching the T2WI images in axis plane. Sequence parameters were as follows: repetition time/echo time, 2868/108 milliseconds; b-values, 0 and 1000 s/mm²; diffusion directions, 6; FOV, 160 mm×160 mm; matrix, 92×92; averages, 2; slice

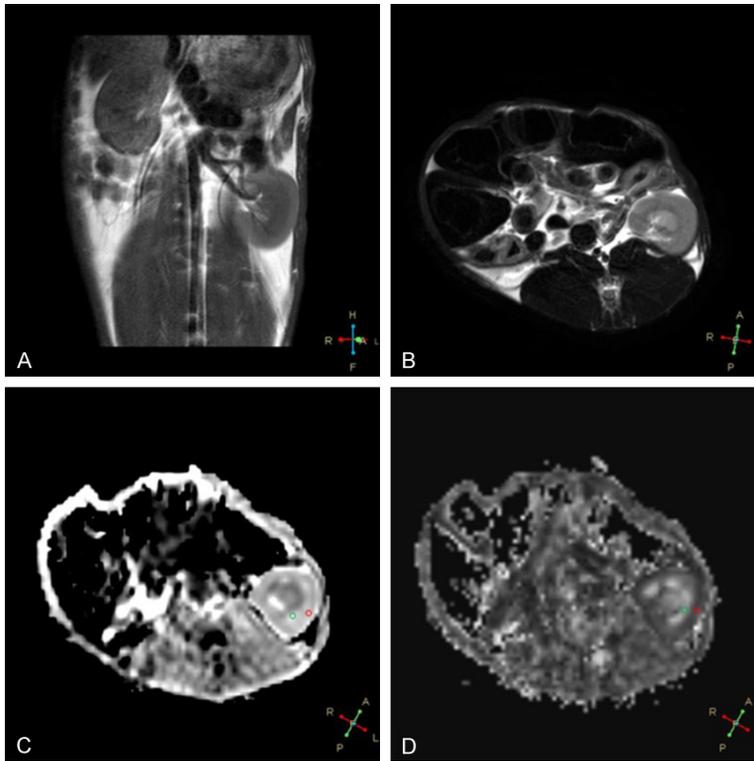


Figure 1. MRI images of the rabbit. A-D: T1, T2, ADC and FA maps. Freehand regions of interest (ROIs) were positioned in the middle part parenchyma of the ADC and FA image, and we draw 4-6 ROIs in each slice. Red ROI was located in cortex and green ROI was located in medulla.

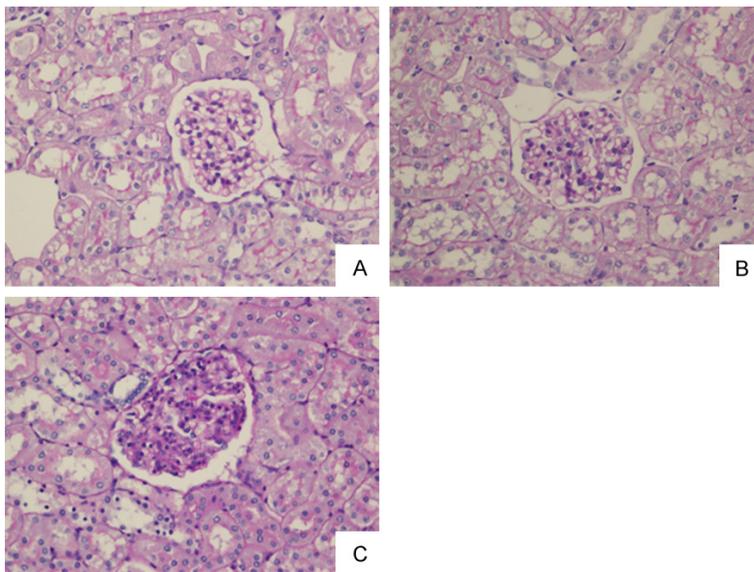


Figure 2. Periodic acid-Schiff (PAS) staining of control and experimental group ($\times 400$). (A) Image of a control kidney, there is no mesangial cells proliferation. (B) Image of an experimental kidney for 4 weeks reveals glomerular changes, such as mesangial expansion, glomerulosclerosis and glomerular hypertrophy when compared with controls (A). (C) Image of an experimental kidney for 8 weeks reveals significant mesangial cells proliferation.

thickness, 3 mm; slice gap, 0; acquisition time, 5 minutes.

MR data analysis

An MRI workstation (EWS 2.6.3.3; Philips) was used to analyze the images. Two abdominal radiologists with expertise in abdominal MRI, and blinded to histopathological findings and experimental technique, evaluated the DTI images (**Figure 1**). Image quality of FA and ADC-maps was assessed in distortion, motion artifacts, corticomedullar discrimination. A 5-point-scale was applied (5 = very good; 1 = poor). The degree of diffusion anisotropy was calculated and depicted in parameter maps of FA, which are scaled from 0 (no preferred diffusion direction, isotropic diffusion) to 1 (only 1 diffusion direction, completely anisotropic diffusion). Apparent diffusion coefficient maps were calculated based on a monoexponential fitting model. The free-hand regions of interest (ROI) were positioned in the parenchyma of mesorenal area, as the evaluation of ADC values in the central portion of the kidneys is reliable and suitable for use in longitudinal studies [12].

Histology

For assessment of glomerulosclerosis, 20 representative glomeruli of each animal were evaluated, and mesangial cell number and the percentage of PAS positive area per total glomerular tuft area were counted (**Figure 2**).

Statistical analysis

Statistical analyses were performed using statistical pack-

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Table 1. mean renal ADC values and FA value of three groups

	ADC value of cortex (10^{-3} mm ² /s)	rADC of cortex	ADC value of medulla (10^{-3} mm ² /s)	rADC value of medulla	FA value of cortex	FA value of medulla
Group A	1.939±0.210	1.728±0.181	1.759±0.083	1.447±0.096	0.303±0.034	0.546±0.059
Group B	1.832±0.134	1.669±0.132	1.702±0.094	1.417±0.084	0.265±0.050	0.575±0.070
Group C	1.674±0.265	1.519±0.241	1.642±0.037	1.422±0.151	0.219±0.044	0.545±0.067
F	5.968	4.659	6.387	0.299	15.043	1.032
P	0.005	0.015	0.004	0.743	<0.001	0.365

ADC and rADC of the cortex was significantly higher than that of the medulla. FA of the cortex was significantly lower than that of the medulla in all measurements. The FA, ADC and rADC of the cortex was gradually decreased.

age for the social sciences version 19.0 (SPSS Inc., Chicago, USA). Values are expressed as mean \pm standard deviation. Independent-samples t test was used to compare mean value between cortex and medulla in each group. One-way ANOVA test was used to determine significant differences in mean ADC and mean FA values between groups. Post-hoc comparison Student-Newman-Keuls test was used to compare subgroups. A *P* value of less than 0.05 was considered statistically significant for all analyses.

Results

MRI results

The average image scores of FA and ADC images were 4.3 and 4.8. Intra-reader correlation for the quality of FA and ADC image was excellent with kappa-values 0.72 and 0.86, respectively. Image quality was well enough for diagnosis. There was no significant difference between cortical/medulla value of different time point about ADC and FA in control group, so we combined the value in control group of different point as group A. Then the date in group A was 14 (After MRI exam at 4 weeks we executed 2 rabbit). We divided the model group into 2 groups, 4 weeks (group B) and 8 weeks (group C) after model, respectively. **Table 1** shows mean renal ADC values, rADC value and FA value of three groups. The cortical relative apparent diffusion coefficient (rADC) value was higher than medullary in all three groups. There were statistical differences between cortical rADC and medullary rADC value in group A ($P < 0.001$), and so was in group B ($P < 0.001$). rADC values of cortex and medulla of groups B and C decrease compare to group A. There were statistical differences between groups A

and C in cortical rADC value ($P = 0.005$), as well as between groups B and C ($P = 0.038$). There were no significant differences among the three groups in medullary rADC value.

The cortical fractional anisotropy (FA) value was lower than medullary in all three groups and there were statistical differences in group A ($P < 0.001$), group B ($P < 0.001$) and group C ($P < 0.001$). Cortical FA values of group B and group C were gradually decreased compared to group A. There were statistical differences between groups A and C in cortical FA value ($P < 0.001$), group B and group C ($P = 0.004$), as well as between groups A and B ($P = 0.017$). There was no statistical difference in medullary FA value among the three groups.

Histopathology

One can found glomerular mesangial cells proliferation in all executed model rabbits. The glomerular of control group was normal. There were less than 3 mesangial cells in each capillary mesangium region of group B. But there were more than 3 mesangial cells in each capillary mesangium region of group C, especially mesangial diffuse hyperplasia and aggregation; capillary structure damage could be seen in some mesangium region.

Discussion

The ADC value is a quantitative parameter computed from the DW MR images, which reflects the influence of water diffusion in the extracellular extravascular space and capillary perfusion. Notohamiprodjo M suggested that movement of blood in microvasculature could be modeled as a pseudo-diffusion process, which is measurable at low *b* values (< 200 s/mm²) [13]. High *b* values have been generally pre-

ferred for tumor detection to minimize T2 shine-through and perfusion effects within the capillary networks. However, a higher b value leads to a lower signal to-noise ratio (SNR). But at 3T, signal-to-noise ratios (SNR) between cortex and medulla and contrast-to-noise ratios (CNR) between cortex and medulla were significantly higher than those at 1.5T, leading to improved corticomedullary discrimination [12, 14, 15]. For our study, the b values were set at 0 and 1000 s/mm² to maintain SNR.

In this study, the most classic heterologous immunity protein intravenous method was applied for mesangial proliferative glomerulonephritis model, which is molded high, reproducible and relatively simple. All animal were successfully modeled. The renal hilum plane is selected for measurement [12], since the volume and flow of glomerular arterioles are increased compared with bipolar. Therefore, cortical ADC value is larger than the bipolar of the kidney. Tubule of renal medulla in the region of poles fluid flow velocity and flow rate is less than the area near the renal papilla, and the renal hilum plane containing a complete kidney cone, so the renal hilum medulla ADC value is larger than the upper and lower poles. In addition, the area of middle level was the largest and suitable for accurate interest area set. In all measurements, ADC of the cortex was significantly higher than that of the medulla, whereas FA of the cortex was significantly lower than that of the medulla, which are in line with renal anatomy and other studies [3, 7, 8, 13]. The reason is probably high renal cortical blood perfusion and the radial organization of the tubules and the collecting ducts, draining into the renal pelvis.

MsPGN characterized as mesangial cell proliferation and mesangium extracellular matrix expansion, meanwhile often accompanied with tubulointerstitial changes, probably due to insufficient blood supply caused by glomerular lesions. The ADC value and FA value of cortex gradually decrease with increasing severity of renal pathology in our study, which is also in line with previous studies [8, 16]. Because pathological changes such as mesangial cell proliferation, glomerulosclerosis, tubular atrophy, and interstitial fibrosis reduce renal water content, perfusion, and the extracellular diffusion of water molecules, and thereby decrease the renal ADC value [17]. The reduction in diffu-

sion anisotropy is also caused by renal pathologies impair diffusion along the radial oriented tubules [18, 19]. This experiment was an early model of MsPGN disease, characterized with glomerular lesions; hence ADC value reduction of cortex is more obvious than medulla.

The current study has several inherent limitations that warrant mention. First, the region of interest for the value measurement was manually derived from the central section of the kidney and might not match the biopsy site, which implies a certain degree of subjectivity and bias. Second, we did not use biexponential intravoxel incoherent motion model (IVIM) for the analysis of DWI, which may provide a better fit to the diffusion-weighted signal than the monoexponential analysis [5, 20].

In summary, DWI has potential for diagnosis of mild MsPGN and assessing the severity of renal pathology and is a non-invasive and effective technique for guiding therapy and follow-up in MsPGN patients.

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Disclosure of conflict of interest

None.

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