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Correlation of Diffusion-weighted MR Imaging with Cellularity of Renal Tumours

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Abstract. *Background: Diffusion is a physical process based on the random movement of water molecules known as Brownian movement. Diffusion-weighted imaging (DWI) is a magnetic resonance (MR) technique that provides information about the biophysical properties of tissues such cell organization and density, microstructure and microcirculation. Materials and Methods: Twenty healthy volunteers and 18 patients with renal tumor were enrolled in our study. The DWI was obtained before contrast media injection with a single-shot SE EPI Inversion Recovery (IR) sequence. The tumor cellularity of each resected lesion was evaluated. Results: The mean apparent diffusion coefficient (ADC) value of renal tumors was significantly lower than the mean ADC value of normal renal parenchyma. In our series, the mean ADC value of renal tumors did not significantly correlate with tumor cellularity, but correlated with histological architecture. Conclusion: These preliminary results indicate the utility of DWI in the acquisition of tissue characterization data of renal masses using a minimal acquisition time (17 sec).*

Diffusion–weighted (DW) magnetic resonance imaging is sensitive to molecular diffusion since the random motion of water molecules in the field gradient produces incoherent phase–shifts that result in signal attenuation.

Magnetic resonance imaging (MRI) is the only method today to evaluate the molecular diffusion process *in vivo*. Diffusion-weighted imaging (DWI) is a MR technique that provides information on the biophysical properties of tissues such as cell organization, cell density, microstructure and microcirculation (1).

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Rapid changes in diffusion characteristics can be observed by calculating the diffusion coefficient (D). However, in a biological system the apparent diffusion coefficient (ADC) is usually used as a diffusion measurement instead of the diffusion coefficient, because the latter depends on factors other than Brownian motion, such as microcirculation.

Until recently, DWI techniques were widely employed in the field of neuroimaging for the evaluation of cerebral ischemia and stroke, multiple sclerosis, and also in the differentiation between normal and tumor tissue. For the abdominal region, DWI has not yet entered common clinical practice as many technical and patient-related difficulties have to be overcome. Respiratory movements, the magnetic susceptibility of air in the lungs and bowel, and movement and pulsatility artifacts are the main causes of poor image quality in abdominal DW-MRI. However, with the advent of the ultrafast single-shot echo-planar imaging technique (EPI) in conjunction with single breath-hold imaging, abdominal DWI has become feasible (2). The acquisition time of the EPI sequences (17 sec) is very fast and this minimizes the effects of gross physiological movement.

The kidney is a particularly interesting organ to study by means of the ADC values because of its high blood flow and water transport functions. Recent studies have already shown the potential value of this method in the evaluation of various renal diseases, such as renal infection, renal ischemia, pionephrosis and diffuse renal diseases (3-5).

The aim of our study was to evaluate the ADC with MRI in normal renal parenchyma and renal tumors and to correlate the ADC values with tumor cellularity and histological architecture.

Materials and Methods

Patients. Twenty healthy volunteers (10 males and 10 females; mean age of 50 years, range: 30-72 years) and 18 patients (10 males and 8 females; mean age of 62 years, range: 29-85 years) with renal neoplasm, previously diagnosed either by ultrasound or computed tomography, were enrolled in the study. The histological

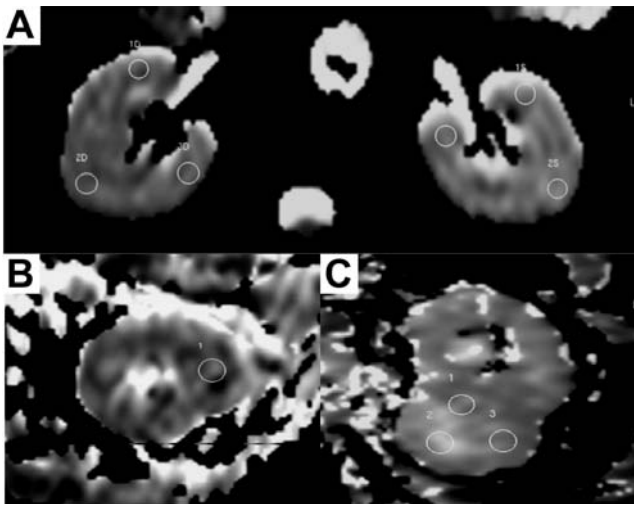

Figure 1. *ROI position choice for ADC value analysis in normal parenchyma (A) and in masses with less (B) or more (C) than 3 cm transverse maximum diameter.*

characterization of the lesions after surgical resection was: 9 conventional (clear cell) renal carcinomas, 2 multilocular cystic renal cell carcinomas, 1 granular cell renal carcinoma, 4 angiomyolipomas and 2 oncocytomas.

MR study. MR was performed using a 1.5 T superconductive magnet (Philips Gyroscan; Best, The Netherlands) with maximum gradient strength of 30 mT/m and slew rate of 120 mT/m/ms, using a synergy body phased-array coil.

In each subject, three morphological sequences were acquired with a single breath-hold: an axial T2-weighted TSE with fat suppression (SPIR) and a coronal T1-weighted FFE or TSE (17 sec) before and after injection of 0.2 mmol/kg of 0.5 molar gadolinium-DTPA contrast media (Magnevist, Schering; Berlin, Germany) at a flow rate of 2.5 ml/s, followed by 20 ml physiological solution at a flow rate of 2.5 ml/s. DWI was obtained before the contrast media injection with a single-shot SE EPI Inversion Recovery (IR) sequence (TE=61, TR=2883, FA=90°, FOV=320mm, Matrix=128x256, slice thickness=7mm, gap=1mm, 16 slices, b value=0 and 500 sec/mm^2) acquired on the axial plane during a single breath-hold, employing a parallel imaging acquisition technique (SENSE).

Evaluation of ADCs. An ADC map was produced for each slice and a 100-mm^2 diameter Region of Interest (ROI) was positioned to determine the mean ADC values. In healthy kidneys, the ROI was placed in three different locations of the cortico-medullary junction (anterior segment, mid segment, posterior segment) for a total of 120 measurements. In renal lesions, one ROI was employed when the focal lesion had a diameter less than 3 cm and three ROIs when the lesion had a diameter of more than 3 cm. The ROIs were carefully placed avoiding necrotic, hemorrhagic and calcified regions (Figure 1).

The comparison between the ADC values of normal parenchyma and neoplastic lesions was performed using the Student's *t*-test. A *p* value <0.05 was considered statistically significant. The data was processed using the SPSS version 11.0 software.

Tumor cellularity analysis. The tumor cellularity of each resected lesion was evaluated using an optical microscope (Nikon Eclipse E 600). The number of cell nuclei in 10 high-magnification (600 x) fields was determined in areas of the histological specimen, colored with hematoxylin-eosin, where no regressive-degenerative phenomena were observable. The analysis was performed by a uninformed expert histologist.

The ADC values were correlated to tumor cellularity using a simple linear regression analysis. A *p* value <0.01 was considered statistically significant.

Table I. *ADC values (x10^{-3} mm^2/sec) in normal renal parenchyma and renal tumors.*

	Number of cases	Mean ADC values (x10^{-3} mm^2/sec)
Normal parenchyma	20	2.23±0.20
Tumors	18	1.72±0.46

Table II. *ADC (x10^{-3} mm^2/sec) and cellularity (x10hmFOV) value in renal tumors.*

Tumors	Number of cases	Mean ADC values (x10^{-3} mm^2/sec)	Mean cellularity values (x10hmFOV)
Conventional (clear cell) carcinoma	9	1.5±0.8	750
Cystic renal cell carcinoma	2	2.44±0.4	750
Granular cell renal carcinoma	1	2.67	3058
Oncocytoma	2	1.71±0.3	2998
Angiomyolipoma	4	1.46±0.09	1100

Results

ADC. All images were diagnostic and no case was excluded from the study. The mean ADC value of renal tumors of 1.72±0.46 x 10^{-3} mm^2 /sec was significantly lower than the mean ADC value of normal renal parenchyma (healthy volunteers) of 2.23±0.20 x 10^{-3} mm^2 /sec ($p<0.001$) (Table I).

The ADC values of the different histological types ranged between 1.07 x 10^{-3} mm^2 /sec and 2.01 x 10^{-3} mm^2 /sec, with the exception of 2 cases of multilocular cystic renal cell

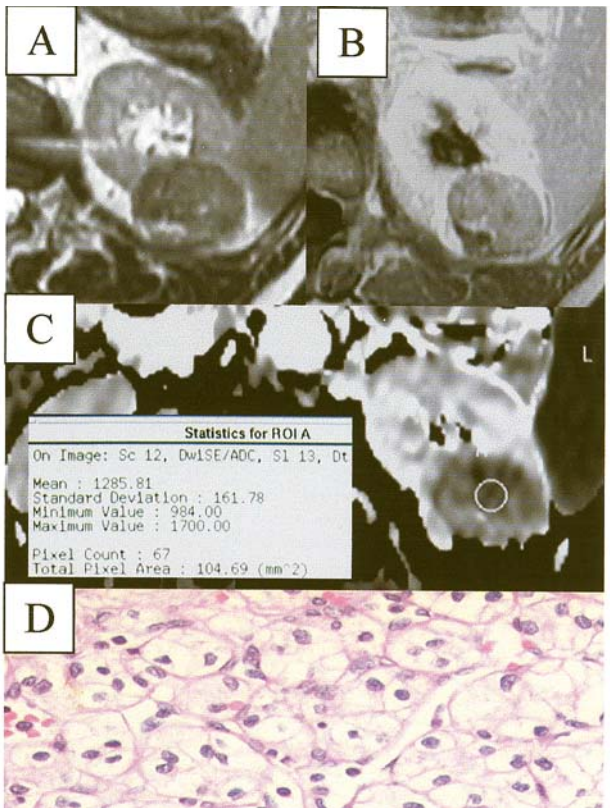

Figure 2. *Multilocular cystic renal cell carcinoma. Axial acquisition TSE T2-weighted (A) and TFE T1-weighted post-Gadolinium (B), ADC map and value (C) and histological specimen (D).*

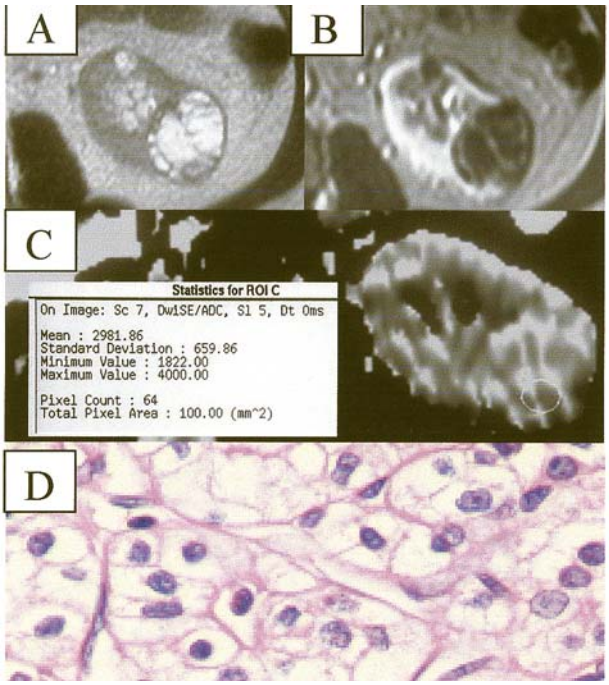

Figure 3. *Granular cell renal carcinoma. Axial acquisition TSE T2-weighted (A) and TFE T1-weighted post-Gadolinium (B), ADC map and value (C) and histological specimen (D).*

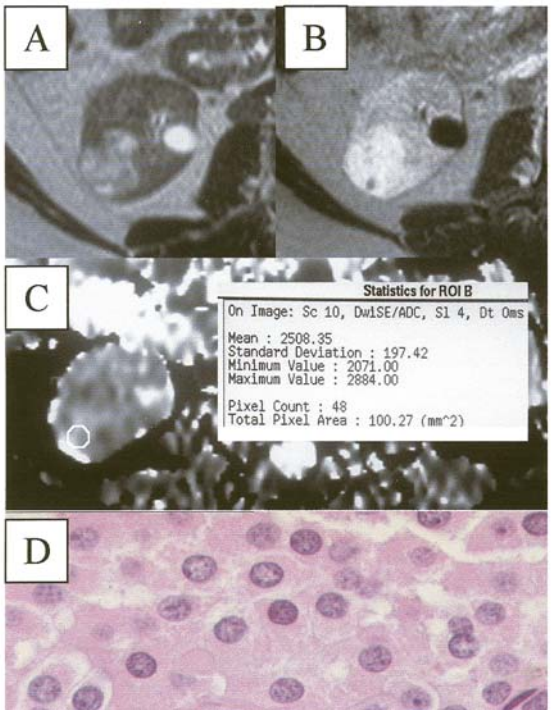

Figure 4. *Conventional (clear cell) renal carcinoma. Axial acquisition TSE T2-weighted (A) and TFE T1-weighted post-Gadolinium (B), ADC map and value (C) and histological specimen (D).*

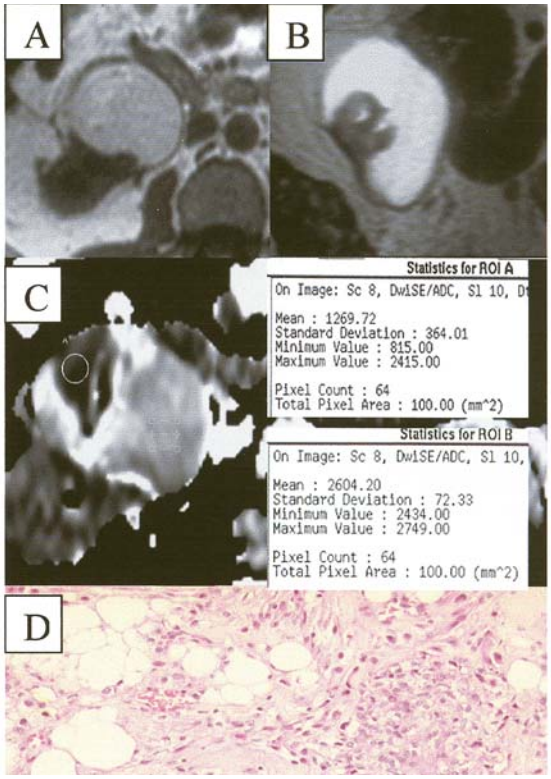

Figure 5. *Angiomyolipoma. Axial acquisition TFE T1-weighted (A), coronal acquisition TSE T2-weighted (B), ADC map and value (C) and histological specimen (D).*

carcinoma (2.51±0.4 x 10^{-3} mm^2 /sec) (Figure 2C) and 1 case of granular cell renal carcinoma (2.67 x 10^{-3} mm^2 /sec) (Figure 3C) that had marginally overlapping ADC values with the normal renal parenchyma (Table II).

Tumor cellularity. The mean number of neoplastic cell nuclei in 10 high-magnification fields was 750 for conventional (clear cell) renal carcinoma (both solid and cystic variants) (Figure 4D-2D), 1800 for renal carcinoma with mixed cellular aspects (association of granular and clear cells), 3058 for granular cell renal carcinoma (Figure 3D), 2998 for oncocytoma and 1100 for angiomyolipoma (Figure 5D).

In our series there was not correlation between the ADC and cellularity values for each histological type: the same ADC value was found in tumor with cellularity values ranging from 750 to 2998, like clear cell carcinoma, angiomyolipoma and oncocytoma; on the other hand, the same cellularity value was found in tumor with different ADC value, such as clear cell carcinoma and cystic renal cell carcinoma, or oncocytoma and granular cell renal carcinoma (Table II).

Discussion

Currently, MRI is considered the only technique capable of measuring *in vivo* molecular diffusion.

Considering its high blood flow and fundamental function of fluid transport and exchange, that justify high ADC values, the kidney is an ideal organ for DWI MR. The mean ADC value of normal renal parenchyma was 2.23±0.20 x 10^{-3} mm^2 /sec.

Several studies, performed in the last 10 years using different MR techniques, report discordant ADC values in human kidney. The variability of the data is due to the different diffusion weightings used for the sequence, represented by the *b factor* value, encompassing the main characteristics of diffusion gradients (amplitude, duration and time interval between gradients) (2,3,6,7).

The ideal diffusion weighting of an echoplanar sequence is still problematic. When low *b factor* values (30-100 s/mm^2) are used, the perfusion of the microcircle and the T2 tissue components determine an overestimation of ADC values. On the other hand, the use of high *b factor* values (>1000 s/mm^2) cause the formation of extremely noisy images due to the low MR signal generated by the short T2 time of abdominal tissues. We believe that the diffusion sequence of our study (TE 61 msec, *b factor* 500) represents a valid compromise between high diffusion weighting and final image quality.

The measurement of ADC values was performed placing the ROI in the cortico-medullary junction of the mid segment due to its lower dependence on perfusion effects (2).

Statistically significant differences were found between normal renal parenchyma and renal tumor ADC values, with a marginal overlapping of ADC values only in the case of two multilocular cystic variants of conventional (clear cell) renal carcinoma and one granular cell renal carcinoma.

In our series, the mean ADC value of renal tumors did not correlate to tumor cellularity with statistical significance, but correlated to histological architecture, demonstrating how extracellular water diffusibility can represent sensitive and specific data for the classification of renal masses. The standard deviation of the mean ADC values results were proportional to tissue inhomogeneity (Table II). This is evident in conventional renal carcinoma, which grows forming uniform and homogenous solid cell sheets. In our study, this histotype yielded a cellularity of 770 in 10 high-magnification fields of view (8 cases) and a mean ADC value of 1.56±0.2 x 10^{-3} mm^2 /sec. Only in one case of a mixed cellularity renal cell carcinoma (clear and granular cells) was the same ADC value correlated to a tumor cellularity of 1800. This can be explained by the particular histological growth modality of this variant, characterized by hypercellular pseudopapillary projections constituted of pseudopapillae without a true fibrovascular core, separated by interstitial spaces where water can spread freely.

Different considerations have to be made for granular cell renal carcinoma and oncocytoma. These tumors form trabecular structures, nests and tubular formations containing large interstitial spaces where water spreads freely. In fact, due to their identical histological and histochemical properties, differential diagnosis between these tumors is extremely difficult and can only be performed through determining the number of cytoplasm mitochondria by electron microscopy. According to our study, granular cell renal carcinoma and oncocytoma present very high cellular densities (3058 and 2998 in 10 high-magnification fields of view, respectively) and different mean ADC values (2.67 x 10^{-3} mm^2/sec and 1.71 x 10^{-3} mm^2/sec, respectively). This observation, if confirmed by large series, would be extremely important diagnostically.

Angiomyolipoma, although composed of differing amounts of smooth muscle, adipose tissue and vasculature with a significant cellularity (1100), contains a collagenous interstitial stroma which reduces water diffusion velocity, thus resulting in low ADC values (1.46±0.09 x 10^{-3} mm^2 /sec) that overlap with the ADC values of renal cell carcinoma. The two lesions can be easily distinguished with conventional MR sequences.

In conclusion, these preliminary experiences indicate the utility of DWI MR in the characterization of neoplastic renal masses on the basis of cellularity, representing an indirect indicator of the complexity of intercellular spaces and junctions, histological architecture, free and bound water content and the tissue's growth modality. Using a

minimal acquisition time (17 sec), this technique can supply tissue characterization data not otherwise available with other non-invasive techniques. DWI MR could be introduced in clinical practice as an integration of standard MR protocols employed for the kidney; nevertheless, the validation of the differences existing between the ADC values of the different renal tumors and the definition of the clinical applications of this technique require further investigations.

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