

Original Article

Histological grade of hepatocellular carcinoma predicted by quantitative diffusion-weighted imaging

Weihua Guo, Suhong Zhao, Yuhai Yang, Guangrui Shao

Department of Radiology, The Second Hospital of Shandong University, Jinan 250033, Shandong, China

Received January 6, 2015; Accepted February 21, 2015; Epub March 15, 2015; Published March 30, 2015

Abstract: Objective: This study is to evaluate the application of diffusion-weighted imaging (DWI) in determining the histological grade of hepatocellular carcinoma (HCC). Methods: Totally, 27 HCC patients who received DWI examination before surgical resection were included in this study. Relationships of apparent diffusion coefficient (ADC) and signal intensity (SI) with the histological grade of HCC were analyzed. Results: These 27 HCC patients could be classified into 6 well, 10 moderately, and 11 poorly differentiated HCCs. The overall ADC value for all HCC cases was $(1.28 \pm 0.19) \times 10^{-3} \text{ mm}^2/\text{s}$. The ADC value for poorly differentiated HCCs was $(1.16 \pm 0.16) \times 10^{-3} \text{ mm}^2/\text{s}$, significantly lower than the well $[(1.43 \pm 0.09) \times 10^{-3} \text{ mm}^2/\text{s}]$ and moderately $[(1.34 \pm 0.19) \times 10^{-3} \text{ mm}^2/\text{s}]$ differentiated HCCs. There was no significant difference in ADC between the well and moderately differentiated HCCs. The overall SI value for all the HCC cases was 75.66 ± 32.94 . The mean SI value for the moderately differentiated HCC cases was 54.37 ± 28.37 , significantly lower than the well (90.78 ± 27.49) and poorly (86.77 ± 31.51) differentiated HCCs. No significant difference in SI was observed between the well and poorly differentiated HCCs. Additionally, there was a significant negative correlation between the ADC value and the histological grade of HCC. Conclusion: The ADC value based on DWI is useful in determining the histological grade of HCC, while the SI value provides limited contribution to HCC histological grade evaluation.

Keywords: Hepatocellular carcinoma, histological grading, magnetic resonance imaging (MRI), diffusion-weighted imaging (DWI), apparent diffusion coefficient (ADC), signal intensity (SI)

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant neoplasm of the liver, with high mortality and poor prognosis, especially in Asia [1]. Conventional treatment options for HCC mainly include local therapies, such as surgical resection, liver transplantation, radiofrequency ablation, and transcatheter arterial embolization [2, 3]. Surgical resection is considered as one of the most effective treatments for HCC. However, the prognosis remains unsatisfactory because of the high recurrence rate. Specifically, compared with well and moderately differentiated HCCs, poorly differentiated HCC is always associated with higher recurrence rate and poorer prognosis after surgical resection [4]. Moreover, histological grade is one of the important prognostic factors of HCC [5, 6]. If the histological grade could be predicted for each HCC case, it would provide great benefit in preoperative treatment planning.

Previous studies concerning the evaluation of the histological grade of HCC mainly focus on hemodynamics and morphology. It has been revealed that arterial blood supply would be decreasing as HCC progressed from moderate to poor differentiation level [7]. Furthermore, the presence of capsule might indicate moderate or poor differentiation levels of HCCs [8].

Diffusion-weighted imaging (DWI) is a functional magnetic resonance imaging (MRI) technique, which is fundamentally different from the conventional MRI. DWI depends on the measurement of water molecule diffusion in tissues. Apparent diffusion coefficient (ADC) could quantitatively reflect the diffusion characteristics, with low ADC values suggesting restricted diffusion. DWI has originally been used in neuroradiology as a diagnostic tool. In recent years, with the rapid development of magnetic resonance technology, the clinical application of DWI has been greatly extended to extracranial tumor imaging, including the abdomen [9-12].

There are several reports regarding the application of DWI in differential diagnosis between benign and malignant hepatic tumors, and in assessing the therapeutic effects on related diseases [13-15]. However, the relationship between the histological grade of HCC and DWI assessment has not yet been fully established [16-19]. In this study, we investigated whether the histological grade of HCC could be estimated according to the ADC and SI values based on DWI before hepatic resection.

Materials and methods

Patients

This study included totally 27 patients with HCC, 24 males and 3 females, aged 35-66 years (with a mean age of 55.9 ± 9.5 years), who underwent surgical resection in our hospital, from July 2008 to June 2013. The inclusion criteria were as follows: (1) none of the tumors had received clinical treatments before surgical resection; (2) subjects underwent both conventional MRI and DWI examinations before surgery; (3) these were no artifacts in DWI results; (4) tumors were pathologically confirmed as HCC; and (5) tumor size was equal to or larger than 1.0 cm. Out of these 27 HCC patients, 25 cases were with a single HCC, while the other 2 cases were with two lesions, in which the larger lesion was chose for the evaluation. The mean tumor size was 5.6 ± 2.6 cm along the long axis, ranging from 2 to 18 cm. Hepatitis B virus were detected in 25 patients, and hepatitis C virus were detected in the other 2 patients. The time interval between examination and surgery ranged from 5 to 12 d. Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of the Second Hospital of Shandong University.

MRI and DWI examinations

MRI examination was performed with a 3.0T system (GE Signa Excite; GE Healthcare, Milwaukee, WI, USA), equipped with eight-channel phased-array coils. Axial T2-weighted imaging (T2WI) was performed using respiratory-triggered fast spin echo sequence with the following parameters: repetition time (TR), 6500-7000 ms; echo time (TE), 85-90 ms; slice thickness, 7 mm; interslice gap, 1.5 mm; matrix, 320×224 ; field of view, $27 \text{ cm} \times 36 \text{ cm}$; num-

ber of excitation, 4; width of band, 62.5 KHz; acquisition time, approximately 2-2.3 min.

Axial T1-weighted imaging (T1WI) was performed using breath-hold fast spoiled gradient echo sequence, with the following parameters: TR, 230 ms; TE, 2.3 ms; slice thickness, 7 mm; interslice gap, 1.5 mm; matrix, 320×192 ; field of view, $27 \text{ cm} \times 36 \text{ cm}$; flip angle, 80° ; number of excitation, 1; width of band, 62.5 KHz; acquisition time, approximately 19-21 s.

DWI was performed with a breath-hold single shot spin-echo echo-planar imaging sequence, in the transverse plane using parallel technique, with the following parameters: TR, 1400 ms; TE, 72.3 ms; slice thickness, 7 mm; interslice gap, 1.0 mm; matrix, 128×128 ; field of view, $38 \text{ cm} \times 38 \text{ cm}$; number of excitation, 4; acquisition time, approximately 22 s; b value, 0 and 600 s/mm^2 ; directions of diffusion gradients, three orthogonal directions (x, y, and z); acquisition time, approximately 22 s.

Image analysis

MR images were reviewed by two experienced radiologists independently, in a blinded fashion. Each final decision was made only with the agreement between these two radiologists. ADC measurements were performed in the GE workstation software. Regions of interest (ROI) were placed on the DWI images. ADC values were automatically calculated on ADC maps, with the location identical to that of the DWI image. ROI was a circle area of 110-120 pixels, covering the smallest lesion with a diameter of 2.0 cm. According to the lesion size, 2-4 uniform ROIs were placed in the solid parts of other HCCs, avoiding necrotic and hemorrhagic regions as indicated by T2WI and T1WI. In this manner, an averaged ADC value from multiple measurements could be obtained for each lesion. Meanwhile, the SI value for each lesion was measured on DWI images in the same way.

Histological grading

Histological grade of HCC was assessed by H & E staining, and compared with previous pathological report for each subject. The final decision was made only when the agreement was obtained. Histological grades of HCC were classified into well differentiation, moderate differentiation, and poor differentiation, respectively. When differential results were observed within

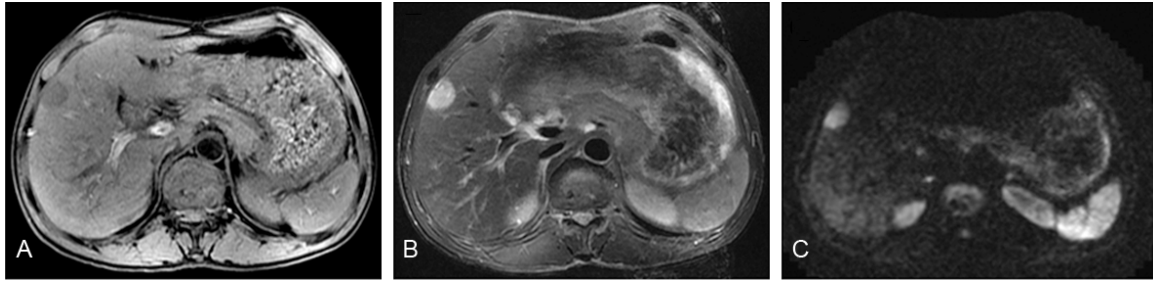


Figure 1. Well differentiated HCC in the right hepatic lobe of a 59-year-old male. Results showed hypointensity on axial T1WI (A), hyperintensity on axial T2WI (B), and hyperintensity on axial DWI (C).

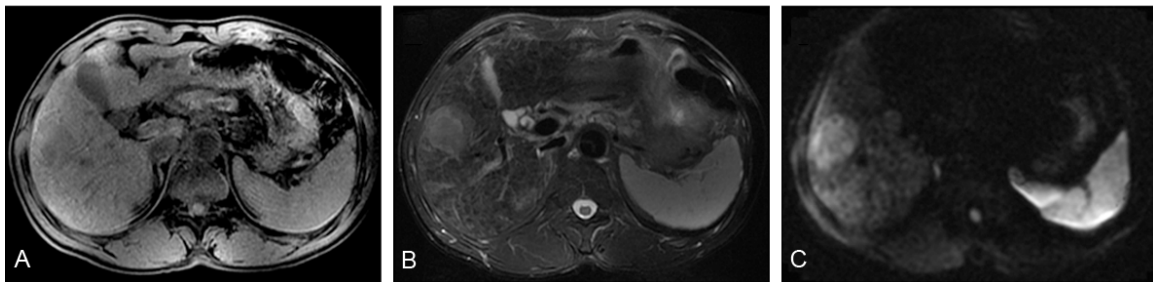


Figure 2. Moderately differentiated HCC in the right hepatic lobe of a 60-year-old male. Results showed slight hypointensity on axial T1WI (A), slight hyperintensity on axial T2WI (B), and hyperintensity on axial DWI (C).

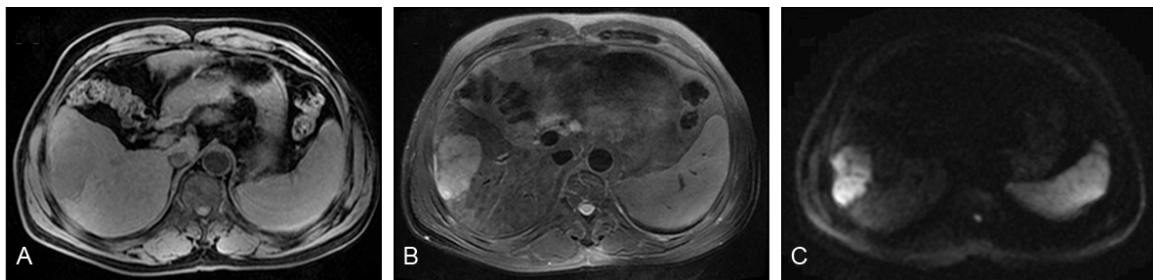


Figure 3. Poorly differentiated HCC in the right hepatic lobe of a 54-year-old male. Results showed hypointensity on axial T1WI (A), hyperintensity on axial T2WI (B), and hyperintensity on axial DWI (C).

the same tumor (e.g., moderate and poor differentiation), the predominant grade would be selected.

Statistical analysis

Data were expressed as mean $\pm$ SD. Statistical analysis was performed using the SPSS16.0 software. One-way ANOVA was performed for the comparison of ADC and SI values, and LSD test was applied for the comparison between histological grades. Pearson correlation test was performed to evaluate the correlation between the histological grade and the ADC value. $P < 0.05$ was considered statistically significant.

Results

Histological grades of HCC

According to the results from histopathological analysis, out of these 27 HCC patients, 25 cases revealed single histological grade, while the remaining 2 cases had two different histological grades (one case mainly with poor differentiation, and the other case mainly with moderate differentiation). Representative MRI and DWI images of cases with well, moderate and poorly differentiated HCCs were shown in **Figures 1-3**, respectively. Overall, these lesions were classified into 6 well, 10 moderate, and 11 poorly differentiated HCCs.

Table 1. Relationships between ADC and SI values with HCC histological grade

HCC histological grade	N	ADC value ($\times 10^{-3}$ mm ² /s)	SI value
Overall	27	1.28 $\pm$ 0.19	75.66 $\pm$ 32.94
Well differentiation	6	1.43 $\pm$ 0.09	90.78 $\pm$ 27.49
Moderately differentiation	10	1.34 $\pm$ 0.19	54.37 $\pm$ 28.37
Poorly differentiation	11	1.16 $\pm$ 0.16	86.77 $\pm$ 31.51
F	-	5.921	4.161
P	-	0.008	0.028

ADC and SI value measurement

The ADC and SI values for HCC cases of each histological grade were shown in **Table 1**. Our results indicated that the overall ADC value for all HCC cases was $(1.28 \pm 0.19) \times 10^{-3}$ mm²/s. Particularly, the ADC value for poorly differentiated HCC cases was $(1.16 \pm 0.16) \times 10^{-3}$ mm²/s, which was significantly lower than the well $[(1.43 \pm 0.09) \times 10^{-3}$ mm²/s; $P < 0.01$] and moderately $[(1.34 \pm 0.19) \times 10^{-3}$ mm²/s; $P < 0.05$] differentiated HCCs, respectively. There was no significant difference in the ADC value between the well and moderately differentiated HCCs ($P = 0.281$). The ADC value decreased when the histological grade of HCC developed. Moreover, there was a significant negative correlation between the ADC value and the differentiation grade of HCC ($r = -0.566$, $P = 0.002$).

On the other hand, the overall SI value for all the HCC cases was 75.66 ± 32.94 . The mean SI value for moderately differentiated HCC cases was 54.37 ± 28.37 , which was significantly lower than the well (90.78 ± 27.49 ; $P < 0.05$) and poorly (86.77 ± 31.51 ; $P < 0.05$) differentiated HCCs, respectively. No significant difference in the SI value was observed between the well and poorly differentiated HCCs ($P = 0.791$).

These results indicate that ADC value could contribute to distinguishing poorly differentiated HCC from well and moderately differentiated HCCs, while SI value only provides limited contribution.

Receiver operating characteristic (ROC) analysis

The diagnostic ability of the ADC value in the histological grade of HCC was evaluated with the ROC analysis. Our results showed that the optimal cutoff point of the ADC value in diag-

nosing poorly differentiated HCC was 1.145×10^{-3} mm²/s. The sensitivity for a cutoff ADC value equal to or less than 1.145×10^{-3} mm²/s in distinguishing poorly from moderately differentiated HCC was 100%, and the specificity was 54.5%.

Discussion

In the present study, quantitative analysis (the ADC and SI values) was performed based on DWI to assess the histological grade of HCC. Our results suggested that DWI could be used to predict the histological grade of HCC before treatment.

There are several reports that have tried to evaluate the relationship between the histological grade of HCC and the ADC value [16-19]. However, discrepancies have been noted. Heo *et al.* [17] have reported that the mean ADC value of poorly differentiated HCCs was significantly lower than the well and moderately differentiated HCCs, suggesting that the ADC value might distinguish between HCCs with different histological grades, which is in line with our results. In general, the histological grade of HCC is determined by cellular and structural atypia. As HCC progresses toward poor differentiation status, it would be characterized by increased cellular density, nuclear/cytoplasmic ratio, and intracellular organelles, as well as thickened cellular plates [20]. These changes might cause the shrinkage of extracellular and intracellular space, followed by the decreased movement of water molecules, eventually leading to restricted diffusion. The more restricted the diffusivity of a tissue is, the lower ADC value it would be obtained.

However, on the other hand, Nasu *et al.* [18] have shown that the histological grade of HCC is not correlated with the ADC value. This finding is not consistent with our results. The contradiction might be due to the different methods used in ADC value measurements. In their study, ROI was selected as large as possible in each lesion, which might also contain necrotic or hemorrhagic regions. It has been reported that the ADC value of necrotic HCC would be significantly increased [21, 22] due to the increased cell membrane permeability. However, in our study, ROI was placed in the

solid part of HCC, deliberately avoiding necrotic and hemorrhagic area. Although a cutoff ADC value could be used to distinguish poorly from moderately differentiated HCCs, there was indeed overlap, which might lower the specificity.

The SI value is much more objective than visual observation in DWI. Visual observation might be largely interfered by the surrounding parenchyma. Our results showed that, even though the SI value could distinguish moderately from well and poorly differentiated HCCs, there was actually no significant difference in the SI value between the well and poorly differentiated HCCs. Since the treatment and prognosis are dramatically different between the well and poorly differentiated HCCs, the SI value would provide limited benefit for the treatment planning for HCC. We speculated that this phenomenon might be due to the T2 shine-through effect. The SI value is dependent on diffusivity and T2-relaxation time, i.e., a lesion with high SI value might result from long T2-relaxation time, rather than the restricted diffusion.

There are several limitations to this study. Firstly, the study population was relatively small. Further studies with large sample sizes are still needed. Secondly, our results were only explained by theoretical derivation, rather than experimental evidence. Thirdly, because ADC may be influenced, at least partially, by perfusion, enhancement patterns of HCC should be also taken into account in future studies.

In conclusion, our results showed that the ADC value based on DWI could be useful in evaluating the histological grade of HCC, while the SI value provided limited contribution to determining HCC histological grade.

Acknowledgements

This work was supported by The Natural Science Foundation of Shandong Province (No. ZR2011HL032).

Disclosure of conflict of interest

None.

Address correspondence to: Guangrui Shao, Department of Radiology, The Second Hospital of Shandong University, No. 247, Beiyuan Road, Jinan 250033, Shandong, China. Tel: 86-138 6910 9935; E-mail: sdshgr@126.com

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