Apparent Diffusion Coefficient Value of Diffusion-Weighted Imaging for Hepatocellular Carcinoma: Correlation with the Histologic Differentiation and the Expression of Vascular Endothelial Growth Factor

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Objective: To evaluate whether the histopathological differentiation and the expression of vascular endothelial growth factor (VEGF) of hepatocellular carcinoma (HCC) do show correlation with the apparent diffusion coefficient (ADC) value on diffusion-weighted imaging (DWI).

Materials and Methods: Twenty-seven HCCs from 27 patients who had undergone preoperative liver MRI (1.5T) and surgical resection were retrospectively reviewed. DWI was obtained with a single-shot, echo-planar imaging sequence in the axial plane (b values: 0 and 1,000 sec/mm^2). On DWIs, the ADC value of the HCCs was measured by one radiologist, who was kept 'blinded' to the histological findings. Histopathologically, the differentiation was classified into well (n = 9), moderate (n = 9) and poor (n = 9). The expression of VEGF was semiquantitatively graded as grade 0 (n = 8), grade 1 (n = 9) and grade 2 (n = 10). We analyzed whether the histopathological differentiation and the expression of VEGF of the HCC showed correlation with the ADC value on DWI.

Results: The mean ADC value of the poorly-differentiated HCCs ($0.9 \pm 0.13 \times 10^{-3}$ mm^2/s) was lower than those of the well-differentiated HCCs ($1.2 \pm 0.22 \times 10^{-3}$ mm^2/s) ($p = 0.031$) and moderately-differentiated HCCs ($1.1 \pm 0.01 \times 10^{-3}$ mm^2/s) ($p = 0.013$). There was a significant correlation between the differentiation and the ADC value of the HCCs ($r = -0.51$, $p = 0.012$). The mean ADC of the HCCs with a VEGF expression grade of 0, 1 and 2 was 1.1 ± 0.17, 1.1 ± 0.21 and $1.1 \pm 0.18 \times 10^{-3}$ mm^2/s, respectively. The VEGF expression did not show correlation with the ADC value of the HCCs ($r = 0.07$, $p = 0.74$).

Conclusion: The histopathological differentiation of HCC shows inverse correlation with the ADC value. Therefore, DWI with ADC measurement may be a valuable tool for noninvasively predicting the differentiation of HCC.

Hepatocellular carcinoma (HCC) is the most common primary malignant neoplasm of the liver and it has high mortality and morbidity rates. The treatment of choice for HCC is surgical resection and liver transplantation (1). Despite that the surgical treatment for HCC has improved, the prognosis remains unsatisfactory because of a high incidence of recurrence related to tumor metastasis and invasiveness (2). Among the various prognostic factors of HCC, poor histological differentiation of HCC, which is related to the risk of recurrence, is a poor prognostic factor (3, 4).

Vascular endothelial growth factor (VEGF) is one of the most important angiogenesis factors that are involved in the development of HCC and it is considered to be associated with the histological differentiation of HCC. The increased VEGF expression of HCC is associated with a poor prognosis due to its association with a higher incidence of microvessel invasion and metastasis (3, 5–6). There have been studies about the conventional MR imaging findings according to the histological differentiation and VEGF expression of HCCs (7–10). With regard to the histological differentiation, HCCs with a higher Edmondson-Steiner grade and poor differentiation showed greater hyperintensity on T2-weighted images than do well-differentiated HCCs with a lower Edmondson-Steiner grade (7–9). Concerning the VEGF expression of HCC, the lesion-to-liver contrast-to-noise ratio on the T2-weighted MR images of an HCC with an intense VEGF expression was significantly higher than the ratio for an HCC with no, weak and moderate VEGF expression (10). In addition, VEGF is referred to as 'vascular permeability factor' and it raises the permeability of blood vessels. Increased vascular permeability as regulated by VEGF can predispose a tumor to increased free water in the extracellular spaces of HCC and so this can affect the motion of the extracellular water molecules (11).

Diffusion-weighted MR imaging (DWI) enables noninvasive characterization of biological tissues based on the properties of water diffusion. DWI has been applied for detecting and characterizing tumors by the use of the apparent diffusion coefficient (ADC) value (12). In addition, this technique is so quick to perform that it can be incorporated into a standard clinical protocol. Although there have been several DWI studies that have focused on the relationship between the histological differentiation and the ADC value of head and neck cancer (13–15), there have been few reports that have examined the relationship between the ADC values and the histological or biological factors in patients with HCC (12, 16). The purpose of this study was to evaluate whether the histopathological differentiation and the expression of VEGF of HCC are correlated with the ADC values on DWI.

MATERIALS AND METHODS

Study Population

This retrospective study received approval of the local institutional ethics committee, and written informed consent was obtained from all the patients. Between July 2005 and July 2007, 47 patients with pathologically confirmed HCCs underwent conventional MRI and DWI. We excluded 20 patients with the following conditions: eight patients had not undergone surgical resection, three had undergone preoperative transarterial chemoembolization, five had a HCC smaller than 3 cm in diameter and the ADC value could not be measured, and four patients had poor DWI image quality due to the location of tumor in the hepatic dome. Consequently, 27 patients were finally included in the study (23 men and four women, mean age: 57 years, age range: 35–73 years). Of the 27 patients, 20 patients had type B viral hepatitis and seven patients had type C viral hepatitis. The clinical severity and progression of cirrhosis, which were evaluated using the Child-Pugh classification, was grade A in 21 patients and grade B in six patients. The types of tumor resection were wedge resection in five patients, bisegmentectomy in one, segmentectomy in nine, a left lateral sectionectomy in two, a right posterior sectionectomy in two and hemihepatectomy in eight patients. The number of resected tumors was a single HCC in 24 patients and multiple lesions in three patients. For the patients with multiple lesions, the largest lesion was selected for evaluation. Twenty-seven HCCs of 27 patients were evaluated. The size range of the 27 HCCs was from 3 to 11 cm in the maximum diameter (mean: 5.6 ± 2.6 cm). Nineteen HCCs were located in the right

Table 1. Patient's Characteristics (n = 27)

Characteristics	Values
Mean age (y)*	57 ± 10.5
Gender	
Men	23
Women	4
Type of underlying hepatitis	
Type B viral hepatitis	20
Type C viral hepatitis	7
Child-Pugh classification	
Grade A	21
Grade B	6
Grade C	0
Type of tumor resection	
Wedge resection	5
Segmentectomy	9
Bisegmentectomy	1
Sectionectomy	4
Hemihepatectomy	8
Resected tumors	
Single	24
Multiple	3
Location of tumor	
Right lobe	19
Left lobe	8
Mean size of resected tumor (cm)*	5.6 ± 2.6

Note.— *Data are mean ± standard deviation, and other values are numbers of patients.

hepatic lobe and the remaining eight HCCs were in the left lobe. The time interval between the liver MRI and surgical resection was 1–35 days (mean: 11 days). Table 1 shows an overview of the characteristics of the patients.

MRI

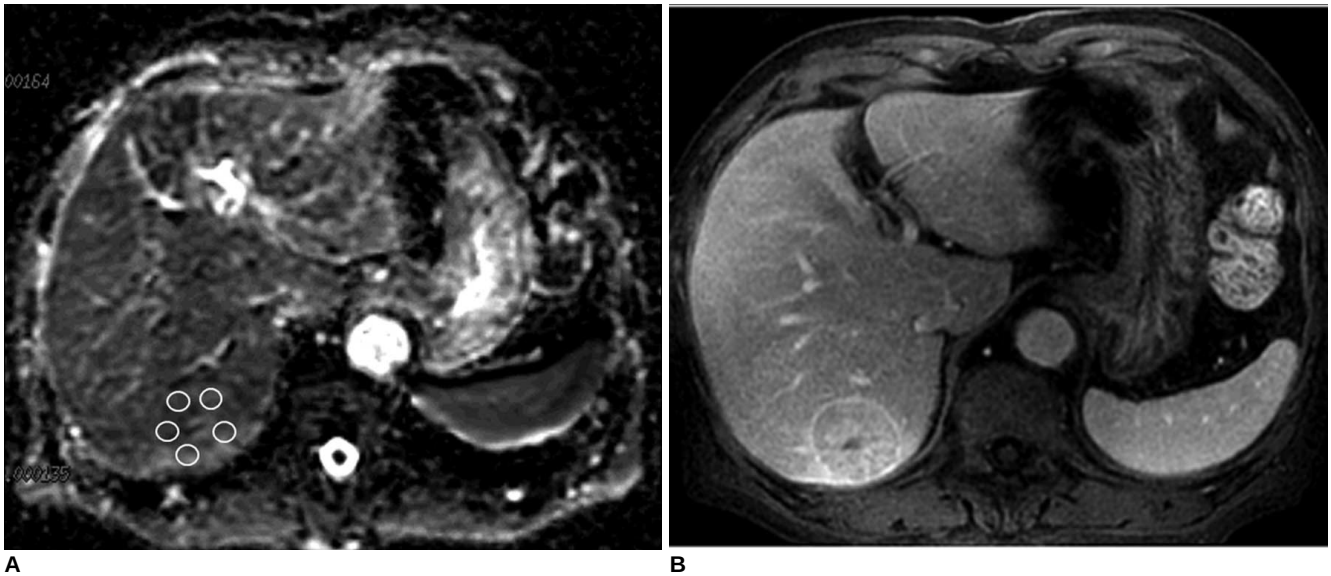
MRI was performed with a 1.5T system (GE Signa Excite GE Healthcare, Milwaukee, WI) with a phased-array multicoil for the body. The MRI protocols included the T1-weighted, T2-weighted, DWI and contrast-enhanced T1-weighted imaging.

In-phase (TR/TE, 125 ms/4.5 ms) and opposed-phase (TR/TE, 125 ms/2.2 ms) T1-weighted spoiled gradient-recalled echo (GRE) sequences were obtained in the axial plane with the following parameters: a slice thickness of 5 mm, an interslice gap of 1 mm, a field of view of 34 × 34 cm, a matrix of 256 × 192, a flip angle of 90° and the number of signals acquired was 1. A T2-weighted fast spin-echo (FSE) sequence (TR/TE: 3,000 ms/80 ms) was performed in the axial plane with the following parameters: a slice thickness of 5 mm; an interslice gap of 1 mm, a field of view of 34 × 34 cm, a matrix of 256 × 256 and the number of signals acquired was 2–4. The gadolinium-enhanced spoiled GRE images were obtained before and after administration of 0.1 mmol per kilogram of body weight gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ) with the following parameters: a slice thickness of 5 mm, an interslice gap of 1 mm, a field of view of 34 × 34 cm, a matrix of 256 × 224 and the number of signals acquired was 1.

Diffusion-weighted imaging was obtained with a single-shot, spin-echo echo-planar imaging sequence using a parallel technique in the axial plane with the following parameters: an TR/TE of 8,000 ms/92 ms, a slice thickness of 5 mm, an interslice gap of 1 mm, a field of view of 34 × 34 cm, a matrix of 128 × 128, the number of excitations was 6 and the b values were 0 and 1000 sec/mm^2. The motion-probing gradients with three orthogonal directions (x, y and z) were applied sequentially. The examination time for the acquisition of the DW images covering the entire liver was 3 minutes under free breathing.

Apparent Diffusion Coefficient Measurement

The ADC values were measured on an ADC map by one radiologist with five years of abdominal MRI experience and who was unaware of the histological findings of the HCCs. The ADC value was automatically calculated by a computer program included in the GE workstation software. The HCCs were identified on the T2-weighted FSE images and the contrast-enhanced T1-weighted GRE images. The image of the mid section of an HCC among several images of each series was selected to minimize the partial volume averaging effect and motion artifacts. The ADC value of the HCC was measured on an ADC map, and the slice's location was identical to that of the selected image on the T2-weighted FSE images and the contrast-enhanced T1-weighted GRE images, respectively. Five regions of interest (ROIs) with a uniform size of 42 pixels were placed in the solid portion of the HCCs that corresponded to the enhancing portion identified on the



A B

Fig. 1. Method of apparent diffusion coefficient measurement. Apparent diffusion coefficient values were obtained by using five ROIs with uniform size (42 pixels) on apparent diffusion coefficient map (**A**), placed on area corresponding to enhancing solid portion of HCCs demonstrated on contrast-enhanced T1-weighted image (**B**). ROIs were carefully placed on solid portion to avoid cystic or necrotic portion.

contrast-enhanced T1-weighted GRE image (Fig. 1). The ROIs were not positioned in the cystic or necrotic portion identified on the T2-weighted FSE images and the contrast-enhanced T1-weighted GRE images because this might have an influence on the quantitative data. The mean ± standard deviation (SD) of the ADC values of the HCCs was calculated.

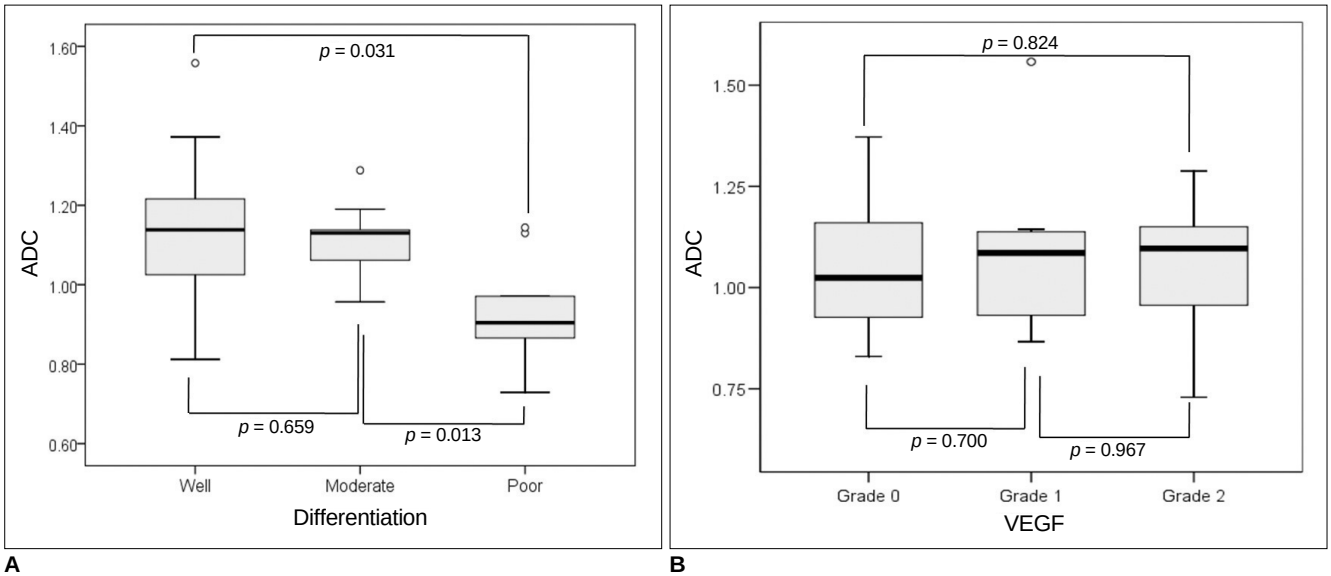
Histopathology

One pathologist with more than 15 years of experience and who was blinded to the MRI findings reviewed the underlying liver disease, the TNM staging, the differentiation and the VEGF expression of the HCCs. For the histopathological and immunohistochemical staining, the tissue block and the field were selected in the mid section of the tumor to correspond as closely as possible to the area where the ADC value was measured on the DWI. Areas of the solid tumor portion were selected with the least amount of non-neoplastic tissue. The Hematoxylin and Eosin stained sections of the formalin-fixed paraffin-embedded tissue were examined with magnifications of ×200 to evaluate the differentiation of the HCCs.

The underlying liver disease and TNM staging were documented on the basis of the American Joint Committee on Cancer (AJCC) classification (17). The underlying liver disease were none to moderate fibrosis (F0: an Ishak score of 0–4) in twenty patients and severe fibrosis or cirrhosis (F1: an Ishak score of 5–6) in seven patients. The T staging of the resected tumors was T1 for sixteen, T2 for eight and T3 for three patients. The N staging was N0 in four patients, N1 in two patients and NX in the remaining 21 patients. The differentiation of an HCC was classified into well, moderate and poor according to Edmondson-Steiner's grading system (18). When different tumor grades coexisted within a tumor, the more predominant differentiation of the tumor was selected.

Immunohistochemistry

Immunohistochemical staining for VEGF was performed on the formalin-fixed paraffin sections by using the avidin-biotin peroxidase complex technique. Briefly, the deparaffinized, rehydrated tissues were placed under steam heating (BioGenex, San Ramon, CA), and this was followed by incubation in 3% hydrogen peroxide for 10 minutes at room temperature to block the internal peroxidase activity. The tissue sections were then incubated with polyclonal antibodies against VEGF (2.0 μg/mL; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:100. The selected section of the tumor was observed with a magnification of ×100 to identify the VEGF-positive cells and to calculate the ratio of the area where positive cells were present to the area of the section examined by one



A **B**

Fig. 2. Graphs of apparent diffusion coefficient value ($\times 10^{-3}$ mm²/s) of hepatocellular carcinomas based on differentiation and vascular endothelial growth factor expression. Outlier indicates range; from largest to smallest observed data points within 1.5 interquartile range presented by box. Horizontal line is median (50th percentile) of measured values; top and bottom of box represent 25th and 75th percentiles, respectively. ADC = apparent diffusion coefficient, VEGF = vascular endothelial growth factor

A. There was significant difference in apparent diffusion coefficient values among well-, moderately- and poorly-differentiated hepatocellular carcinomas (p = 0.026, Kruskal-Wallis test). For pair-wise comparisons, apparent diffusion coefficient value of poorly-differentiated hepatocellular carcinomas was significantly lower than that of moderately-differentiated hepatocellular carcinomas (p = 0.013).

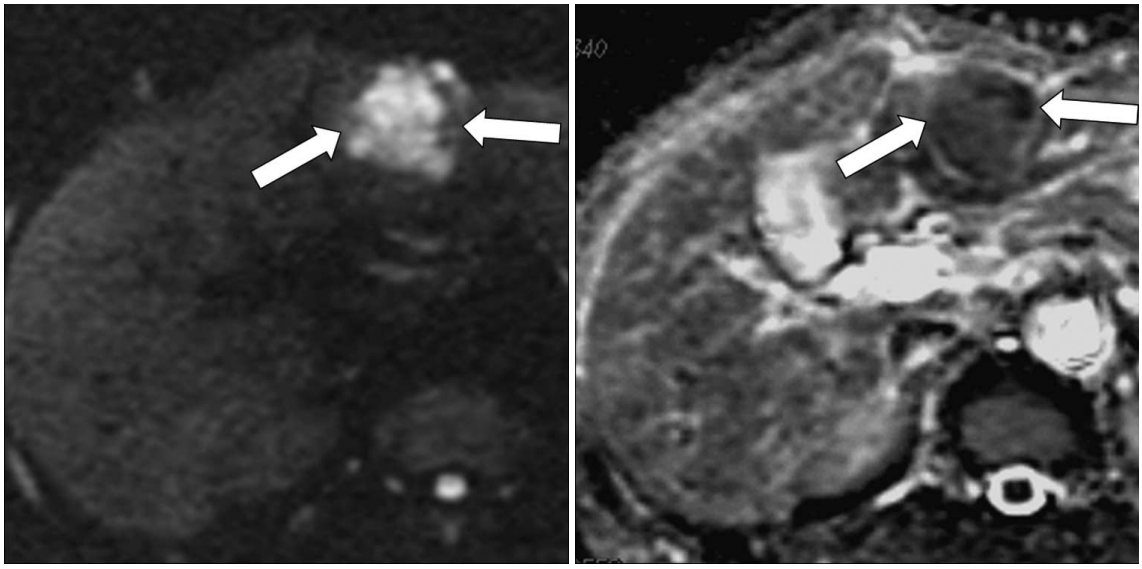
B. There was no significant difference between vascular endothelial growth factor expression and apparent diffusion coefficient value for hepatocellular carcinomas.

pathologist.

The expression of VEGF was semiquantitatively graded into three levels according to the percentage of positive tumor cells in the examined tumor area: VEGF-positive staining HCC cells were present in less than 10% of the examined tumor area for grade 0, between 10% and 50% for grade 1 and greater than 50% for grade 2 (5, 19).

Statistical Analysis

The differences of the ADC values of the HCCs according to the differentiation and VEGF expression were analyzed using the Kruskal-Wallis test. Pair-wise comparisons were performed using the Mann-Whitney *U* test: *p* value less than the Bonferroni-corrected significance value of 0.017 (0.05/3) for all possible pairs was considered to indicate a significant difference. Correlations between the differentiation and the ADC value and between the VEGF expression and the ADC value of the HCCs were evaluated with the Pearson correlation test. Correlation between the differentiation and VEGF expression was also evaluated. The degree of correlation was classified as follows according to the correlation coefficient value (*r*), which was defined as a direct correlation if *r* was a positive value and an inverse correlation if *r* was a negative value: $0 \leq r < 0.25$ was little or no relationship, $0.25 \leq r < 0.5$ was fair, $0.5 \leq r < 0.75$ was moderate to good and $0.5 \leq r < 0.75$ was very good to excellent. $P < 0.05$ was considered statistically significant in all the statistical analyses. All the statistical calculations were performed with commercially available software (SPSS for Windows, release 17.0; SPSS, Chicago, IL).



A B

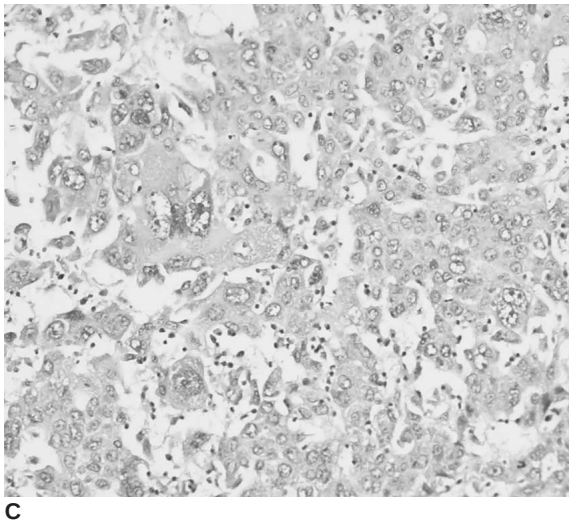


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Fig. 3. 51-year-old man with poorly-differentiated hepatocellular carcinoma.
A. Axial diffusion-weighted image (TR/TE, 8,000 ms/92 ms; and b - 1,000 sec/mm^2) shows 4 × 3.5 cm hepatocellular carcinoma with high signal intensity in left hepatic lobe (arrows).
B. On diffusion coefficient map, apparent diffusion coefficient value of tumor (arrows) was 0.88 ± 0.04 (mean ± SD, $\times 10^{-3}$ mm^2/s).
C. Photomicrograph (Hematoxylin & Eosin staining, ×200) reveals poorly-differentiated hepatocellular carcinoma with irregular nuclei, marked hyperchromatism, and increased mitoses.

RESULTS

All 27 HCCs showed high signal intensity on the DWIs.

For the histological differentiation, 23 HCCs had a single histological grade and four HCCs had two different histological grades. Two of four HCCs were well-differentiated and moderately-differentiated HCC, respectively, and the remaining two HCCs showed a mix of moderate differentiation and poor differentiation. Finally, well-differentiated HCCs were in nine patients, moderately-differentiated HCCs were in nine patients and poorly-differentiated HCCs were in nine patients.

The VEGF expression was grade 0 in eight HCCs, grade 1 in nine HCCs and grade 2 in ten HCCs. The eight HCCs with a grade 0 VEGF expression consisted of three well-differentiated HCCs, two moderately-differentiated HCCs and three poorly-differentiated HCCs. The nine HCCs with a grade 1 VEGF expression were two well-differentiated HCCs, three moderately differentiated HCCs and four poorly-differentiated HCCs. Of the 10 HCCs with a grade 2 VEGF expression, four, four and two HCCs were well, moderately and poorly-differentiated, respectively. There was no correlation between the histological differentiation and the VEGF expression ($r = -0.33$, $p = 0.15$).

Histological Differentiation and the Apparent Diffusion Coefficient Value of the Hepatocellular Carcinomas

The mean ADC value of the well, moderately and poorly differentiated HCCs was 1.2 ± 0.22, 1.1 ± 0.10 and $0.9 \pm 0.13 \times 10^{-3}$ mm²/s, respectively (range: 0.95–1.56, 0.96 – 1.29 and 0.73 – 1.14×10^{-3} mm²/s, respec-

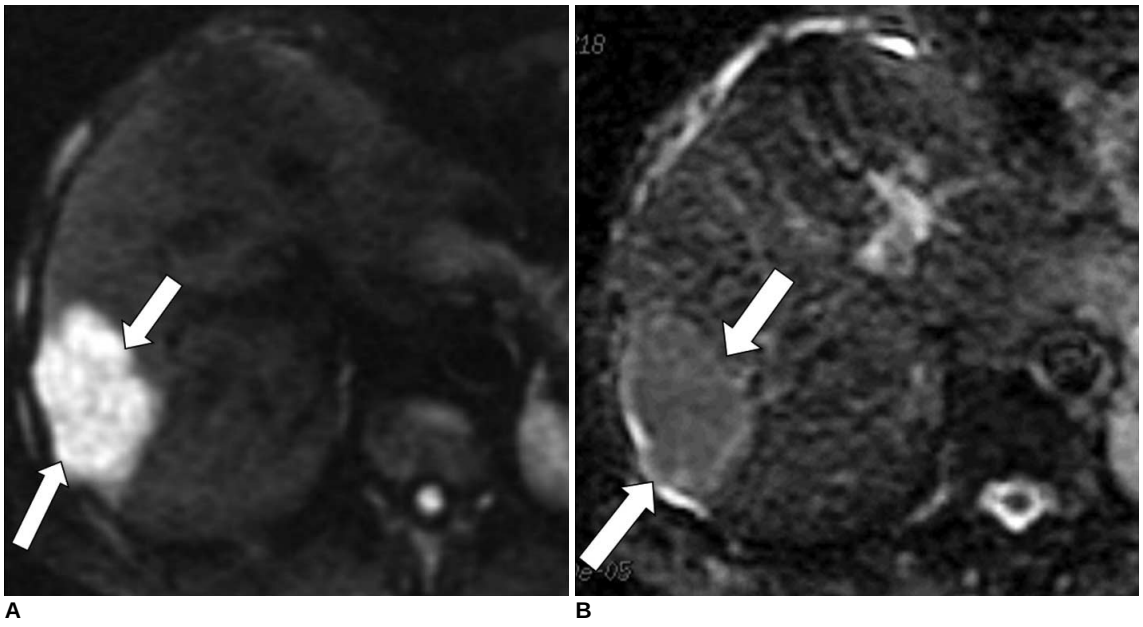

Fig. 4. 63-year-old man with moderately-differentiated hepatocellular carcinoma.
A. Axial diffusion-weighted image (TR/TE, 8,000 ms/92 ms; and b - 1,000 sec/mm²) shows 5 × 3 cm hepatocellular carcinoma with high signal intensity in right hepatic lobe (arrows).
B. On diffusion coefficient map, apparent diffusion coefficient value of tumor (arrows) was 1.14 ± 0.04 (mean ± SD, $\times 10^{-3}$ mm²/s).
C. Photomicrograph (Hematoxylin & Eosin staining, × 200) demonstrates moderately-differentiated hepatocellular carcinoma with round nuclei and relative decrease in mitotic activity.

tively) (Fig. 2A). There was a significant difference in the ADC values among the well-, moderately- and poorly differentiated HCCs ($p = 0.026$, the Kruskal-Wallis test). For pair-wise comparisons, the ADC value of the poorly-differentiated HCCs was significantly lower than that of the moderately-differentiated HCCs ($p = 0.013$) (Figs. 2A, 3, 4). There was no significant difference between the ADC value of the well and moderately-differentiated HCCs ($p = 0.659$) and that of the well and poorly-differentiated HCCs ($p = 0.031$), although the mean ADC value of the well-differentiated HCCs was higher than those of the moderately and poorly-differentiated HCCs (Fig. 2A). The differentiation was inversely correlated with the ADC value of the HCC, and this was significant correlation ($r = -0.51$, $p = 0.012$). An ADC of less than 0.99×10^{-3} mm²/s was found to be the most accurate threshold level for distinguishing HCC with poor differentiation from those with well and moderate differentiation. When this threshold level was applied to our study, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy for predicting HCC with poor differentiation were 78%, 83%, 70%, 88% and 82%, respectively.

Vascular Endothelial Growth Factor Expression and the Apparent Diffusion Coefficient Value of the Hepatocellular Carcinomas

The mean ADC value of the HCCs with grade 0, 1 and 2 was 1.1 ± 0.17, 1.1 ± 0.21 and $1.1 \pm 0.18 \times 10^{-3}$ mm²/s, respectively (range: 0.83–1.37, 0.87–1.56 and 0.73–1.29 $\times 10^{-3}$ mm²/s, respectively). There was no significant difference between the grades of the VEGF expression and the ADC values of the HCCs ($p = 0.90$, the Kruskal-Wallis test) ($p = 0.700$ for grade 0 vs. 1, $p = 0.967$ for grade 1 vs. 2 and $p = 0.824$ for grade 0 vs. 2, the Mann-Whitney U test) (Fig. 2B). The VEGF expression was not correlated with the ADC value of the HCCs ($r = 0.07$, $p = 0.74$).

DISCUSSION

Diffusion-weighted MR imaging is sensitive to several physiological and morphological characteristics of tissue and these characteristics are associated with the slow or fast diffusion of water molecules. DWI with a high b value may be more accurate for estimating the diffusion of water molecules of a tumor in the cellular microenvironment by reducing the contribution of vascular flow, which is affected by such factors as cellularity, the nucleus-to-cytoplasm ratio, the medium of the cytoplasm, biomolecular crowding and the integrity of the cell membrane (20). By performing DWI using different b values, the quantification of water diffusion becomes possible, which is presented as the ADC value. The area with the more restricted diffusion will show higher signal intensity on DWI and a lower ADC value than that of the area with the less restricted diffusion (21).

In our study, the ADC value of poorly-differentiated HCC was significantly lower than that of well- and moderately-differentiated HCC, and the ADC value of the HCCs was inversely correlated with the degree of differentiation. Xu et al. (16) reported that the ADC values of moderately- and poorly-differentiated HCCs were significantly higher than those of the well-differentiated HCCs in a rat model, although there was no significant difference between the ADC value of the moderately- and poorly-differentiated HCCs. The discrepancy between that study and ours could be caused by the different methods to measure the ADC. In our study, ROIs were located on the solid portion to avoid the cystic or necrotic portion on the T2-weighted FSE images and the contrast-enhanced T1-weighted GRE images. However, Xu et al. (16) measured the ADC by using ROIs that encompassed as much of the nodular lesion as possible, and this included the necrotic portion. As tumoral necrosis progresses, the extracellular water tends to be greater and the ADC values of the tumor increase.

The differentiation of the HCCs was classified according to the Edmondson-Steiner grading system, which is mainly dependent on cellular atypia, such as mitotic activity and nucleus/cytoplasm (N/C) ratio that affects the intracellular environment. A poorly-differentiated HCC shows increased mitotic activity, hyperchromatism and an increased N/C ratio (18), which would theoretically decrease the water diffusivity in the intracellular space and result in a reduced ADC value. In our study, a clear correlation ($r = -0.51$) between the differentiation and the ADC value of the HCCs suggests that the differentiation of an HCC is likely to be one of the contributing factors affecting the ADC value. However, Nasu et al. (12) reported that the ADC value of HCCs had no relationship with the histopathologic grade because the current DWI mainly describes the Brownian motion of extracellular water molecules, which conflicts with our result. This discrepancy may be explained by the different b values and methods to measure the ADC. Nasu et al. (12) used an intermediate b value ($b = 500$ sec/mm²) to obtain fine images, but it was not optimized for the ADC measurement, as was described in their limitations. However, DWI with a high b value ($b = 1{,}000$ sec/mm²) was performed in our study, which provides more information about the slow-diffusing water molecules and it can indirectly reflect relatively minor effects such as intracellular microenviron-

ment (21, 22). Additionally, in Nasu et al.'s study (12), the largest possible ROI was created in the slice around the center of the tumor. But we measured the ADC value using five ROIs in the mid section of HCC, which can be less affected by necrosis or a cystic portion.

We hypothesized that increased vascular permeability regulated by VEGF can increase the free water in the extracellular spaces of HCC, resulting in increment of the ADC value. However, there was no correlation in our study for the relationship between the VEGF expression and the ADC of the HCCs, which was not consistent with our assumption. Thus, the relationship between the VEGF expression and the ADC of HCCs on DWI cannot be explained by the role of VEGF as vascular permeability factor. VEGF positivity gradually decreased with the increase of tumor size, and the VEGF expression was even lower in the advanced HCC with increased tumor vasculature (5, 23). Well-differentiated HCCs grow with sinusoidal capillarization controlled by VEGF and they show a higher VEGF expression than do the moderately and poorly-differentiated HCCs (5). Well-differentiated HCCs that are approximately 1.0 to 1.5 cm in diameter would be in a transition stage from the portal blood supply to the arterial blood supply, which would result in an increasing VEGF expression due to the relative hypoxic state from the low blood flow in HCCs at this stage. Therefore, a higher VEGF expression in small and well-differentiated HCCs suggests that VEGF plays an important role in a relatively early stage of angiogenesis of the HCCs. The decrease of the VEGF expression and the presence of well-developed vasculature in the moderately or poorly-differentiated HCCs also indicate the involvement of other angiogenic factors such as basic fibroblast growth factor, angiopoietin or transforming growth factor-β (24). However, our study showed no correlation between the differentiation and the VEGF expression. Furthermore, more than half of the population in our study had moderately- and poorly-differentiated HCCs, and all the HCCs were larger than 3 cm in diameter. Additionally, the small number of subjects and a selection bias to represent the differentiation and VEGF expression in the mid section of tumor should be considered as factors that affected our result between the differentiation and the VEGF expression. Thus, although it remains unclear, these may be the reasons the VEGF expression was not correlated with the ADC value of the HCCs in our study.

This study has several limitations. First, the study population was small. In addition, we selected HCCs that were larger than 3 cm in diameter because we measured the ADC using five ROIs with a 42 pixel size and we tried to avoid overlapping each ROI. The relationship between the histopathological prognostic factors and the ADC values in small HCC should be evaluated in future studies. Second, the MR imaging examination was not exactly coregistered with the surgical specimen. The ADC was measured by using five ROIs in the mid section of the tumor with excluding the necrotic or cystic portions. This sampling bias may also have had a role in contributing to the variations between the ADC values and the histopathologic analysis because this multiple ROI method in one slice may not fully represent the histopathologic heterogeneity of tumor when compared to that of multiple slices. Therefore, further prospective study is needed to match the areas that are histopathologically examined and the areas in which the ADC is measured. Third, DWI was performed with two different b values (0 and 1,000 sec/mm^2) to estimate a more accurate diffusion fraction by minimizing the perfusion fraction to the ADC value. But since multiple b values would enable more precise calculation of an ADC with less perfusion contamination and less regional ADC variations, further study using multiple b-values is also needed. Fourth, the degree of the pattern of enhancement in HCC was not taken into account because we only focused on evaluating the relationship between the ADC value, the differentiation and the VEGF expression. Finally, although there was a significant correlation between the ADC value and the differentiation of HCC, the practical value of the study is limited because there was an overlap of ADC values among each group.

In conclusion, the differentiation of HCC is inversely correlated with the ADC value, which is likely to contribute to affecting the ADC value of HCC. Although correct prediction of the histopathologic grade of HCC is not possible because of the large overlap among the ADC values, DWI with ADC measurement may be helpful for the noninvasive and preoperative prediction of the degree of differentiation of HCC as one of the histological prognostic factors.

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