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EVALUATION OF ASTROCYTOMA CELL PROLIFERATION USING DIFFUSION-WEIGHTED IMAGING: CORRELATION WITH EXPRESSION OF PROLIFERATING CELL NUCLEAR ANTIGEN

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Abstract

The purpose of this study was to analyze if there is a significant correlation between the results of diffusion-weighted imaging (DWI) and the expression of proliferating cell nuclear antigen (PCNA) in astrocytomas. The DWI scans of 19 different-grade astrocytomas were obtained on a 3 T magnetic resonance scanner. The average regional apparent diffusion coefficients (ADC) were measured. The positive expression of PCNA was determined immunohistochemically by using streptavidin-peroxidase complex staining, and was quantified by calculating its calibrated opacity density (COD) using an image analysis system. The average regional ADC and PCNA COD of low grade and high grade astrocytomas were compared. Correlations between regional ADC and PCNA COD were analyzed. The average regional ADC of high grade astrocytomas was significantly ($t = 10.169$, $P = 0.000$) less ($0.687 \pm 0.225 \times 10^{-3}$ mm^2/s) than that of low grade astrocytomas ($1.572 \pm 0.333 \times 10^{-3}$ mm^2/s). The PCNA COD (0.343 ± 0.052) of high grade astrocytomas was significantly ($t=-7.858$, $P=0.000$) greater than that (0.194 ± 0.012) of low grade astrocytomas. There were strong negative correlations between regional ADC and PCNA COD ($r = -0.801$, $P = 0.000$). The results demonstrated that DWI is helpful in evaluating cell proliferation and preoperatively grading astrocytomas by measuring regional ADC.

Keywords

• Astrocytoma • Diffusion weighted imaging (DWI) • Apparent diffusion coefficient (ADC) • Proliferating cell nuclear antigen (PCNA)

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Introduction

Astrocytomas are the most common intracranial tumors. To choose a proper medical strategy and estimate prognosis, it is important to grade the tumor before treatment. The cell proliferation of astrocytoma is proportional to its malignancy. It has been proven that the cellular proliferation of astrocytomas is closely associated with the antigen, proliferating cell nuclear antigen (PCNA) [1-3]. The higher the expression of the antigen, the higher the grade. Diffusion-weighted imaging (DWI) could non-invasively reveal Brownian movement of water molecules of brain tissue. Previous studies have revealed that DWI could be used to grade astrocytomas [4-6]. Although DWI provides insight into the microstructure of the lesion, the relationship between apparent diffusion coefficient (ADC) values and PCNA expression of astrocytomas has not been systematically investigated. The purpose of this study was to investigate whether ADC can reflect the cell proliferation of astrocytomas according to the relationship between ADC and expression of PCNA.

Materials and methods

Patients

Nineteen patients (7 women and 12 men, aged 16-65 years, mean 43.9 years) with pathologically confirmed astrocytomas in Qilu Hospital of Shandong University were enrolled in this study from December 2013 to November 2014. According to the World Health Organization (WHO) classification criteria for central nervous system tumors (2007) [7], there were two pilocytic astrocytomas (WHO grade I), 6 grade II astrocytomas (WHO grade II), 4 anaplastic astrocytomas (WHO grade III) and 7 glioblastomas (WHO grade IV). We defined WHO grade I and II as low grade astrocytomas and WHO grade III and IV as high grade astrocytomas. Therefore, there were 8 low grade astrocytomas and 11 high grade astrocytomas in this cohort. None of the patients had undergone any treatment before DWI examination. All patients were operated on within 7 days after the DWI examination. The study was approved by the institutional review board and informed consent was obtained from all patients.

MR imaging and data processing

The patients' imaging was obtained using a 3.0 T magnetic resonance (MR) imaging system (Signa Excite II; GE Medical Systems, Milwaukee, WI, USA) and an 8-channel phased array coil. The contrast agent was gadopentetate dimeglumine (Magnevist, Schering, Germany). Nonenhanced and contrast-enhanced T1-weighted MR images, T2-weighted MR images, and DWI were

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obtained during the same imaging session without repositioning each patient's head. DWI was performed by using fat-suppressed spin-echo, echo-planar imaging sequence (TR/TE = 6000 ms/minimum; field of view = 23 × 23 cm; matrix size = 128 × 128; 4 excitations; 6-mm slice thickness with 1-mm slice gap) with three orthogonal directional motion-probing gradients (b = 1000 s/mm^2), followed by automatic generation of isotropic DW images. Images without motion-probing gradients (b = 0 s/mm^2) were simultaneously obtained as well.

ADC maps were calculated from isotropic DW images and images obtained with a b value of 0 s/mm^2 by using the Functool 3.1 software on a ADW4.2 workstation (General Electric Medical Systems, Milwaukee, WI, USA). The regional ADC value of each astrocytoma was determined using the following procedures. The solid tumor components with or without contrast enhancement on both conventional MR and diffusion-weighted images were identified in consensus by two neuroradiologists (Q.W., JW.H.) who were blinded to the clinical and histopathologic information. We measured ADC by manually placing 5 to 10 30-40 mm^2 regions of interest (ROI) within solid tumor components on the ADC maps. The average regional ADC values were calculated. The ROI were carefully placed to avoid volume averaging with cystic and/or necrotic areas that would influence ADC values.

Immunohistochemistry

All patients underwent surgical tumor removal. The tumor was resected *en bloc* when possible and by several parts otherwise. All tumors were histologically examined by a neuropathologist. The tissue samples were taken from the solid part of tumors, which meant the tissue was homogenous without necrosis, hemorrhage or cystic change, and immunostained for PCNA. Nineteen samples were fixed in 10% neutral formalin and embedded in paraffin wax. The samples were deparaffinized using dimethylbenzene and ethyl alcohol. Agents including PCNA monoclonal antibody, streptavidin-peroxidase (SP) kit (SP-9000) and 3,3′-diaminobenzidine (DAB) were provided by Beijing Zhongshan Biotech Corp. (Beijing, P. R. China). The immunohistochemistry was performed using SP complex staining according to the instructions provided. PCNA-positive expression was defined as brown particles shown in nuclei. To quantify PCNA expression, Image-Pro Plus version 4.1 image analysis system (Media Cybernetics, Inc., Rockville, MD, USA) was used to detect the calibrated opacity density (COD) of immune reaction products. Ten 100x magnification fields of each slice were selected randomly, and the mean COD was calculated.

Statistics

SPSS 12.0 software was used for statistical analysis. All of the quantitative data was expressed as mean ± standard deviation (SD). Differences in the expression of PCNA and average regional ADC between low and high grade astrocytomas were compared by using an unpaired t-test. Linear regression analysis was performed to assess the relationship between the PCNA COD and average regional ADC. The statistical significance was set at an α level of 0.05.

Results

All patients tolerated the DWI examination and all ADC maps obtained were satisfactory. Table 1 summarizes the clinical and histological

Table 1. Clinical and histological data of 19 patients with astrocytic tumors.

Patient No.	Gender	Age (years)	Tumor location	Pathological diagnosis	Average rADC	PCNA COD
1	Female	36	Left temporal lobe	Astrocytoma II	0.001190	0.1966
2	Male	46	Left frontal temporal lobe	Astrocytoma II	0.001900	0.1807
3	Female	54	Left thalamus	Astrocytoma II	0.001700	0.1810
4	Male	50	Right frontal lobe	Astrocytoma II	0.001330	0.2146
5	Male	41	Right temporal lobe	Astrocytoma II	0.001860	0.1882
6	Male	24	Left frontal lobe	Pilocytic astrocytoma	0.001770	0.1865
7	Male	16	Left cerebellum	Pilocytic astrocytoma	0.001940	0.2030
8	Male	24	Left frontal lobe	Astrocytoma II	0.001530	0.2008
9	Male	57	Left temporal lobe	Glioblastoma	0.000601	0.3973
10	Female	54	Right temporal and occipital lobe	Glioblastoma	0.000353	0.3117
11	Male	52	Left parietal and occipital lobe	Glioblastoma	0.000726	0.3169
12	Female	34	Left frontal lobe and corpus callosum	Anaplastic astrocytoma	0.001030	0.3830
13	Female	32	Right temporal lobe	Anaplastic astrocytoma	0.001200	0.2906
14	Female	48	Left frontal and temporal lobe	Glioblastoma	0.000542	0.2537
15	Male	65	Right frontal and temporal lobe and basal ganglia area	Glioblastoma	0.000651	0.3771
16	Male	48	Left temporal lobe	Glioblastoma	0.000998	0.3573
17	Female	56	Left frontal and temporal lobe	Anaplastic astrocytoma	0.000431	0.3166
18	Male	54	Left frontal and temporal lobe	Glioblastoma	0.000569	0.3257
19	Male	48	Left frontal lobe	Anaplastic astrocytoma	0.000435	0.4343

Abbreviations: COD, calibrated opacity density; PCNA, proliferating cellular nuclear antigen; rADC, regional apparent diffusion coefficient.

data of the 19 patients. The average regional ADC in the 8 low-grade astrocytomas ranged from 1.190 x 10^{-3} to 1.940 x 10^{-3}, with a mean of (1.651 ± 0.277) x 10^{-3}, whereas in the 11 high-grade astrocytomas the average regional ADC ranged from 0.353 x 10^{-3} to 1.200 x 10^{-3}, with a mean of (0.687 ± 0.225) x 10^{-3}. The difference was statistically significant (t = 10.169, P = 0.000, Fig. 1A-C, Fig 2A-C).

The PCNA COD of the 8 low-grade astrocytomas ranged from 0.181 to 0.215, with a mean of 0.194 ± 0.012, whereas in the 11 high-grade astrocytomas the PCNA COD ranged from 0.257 to 0.434, with a mean of 0.343 ± 0.052. The difference was statistically

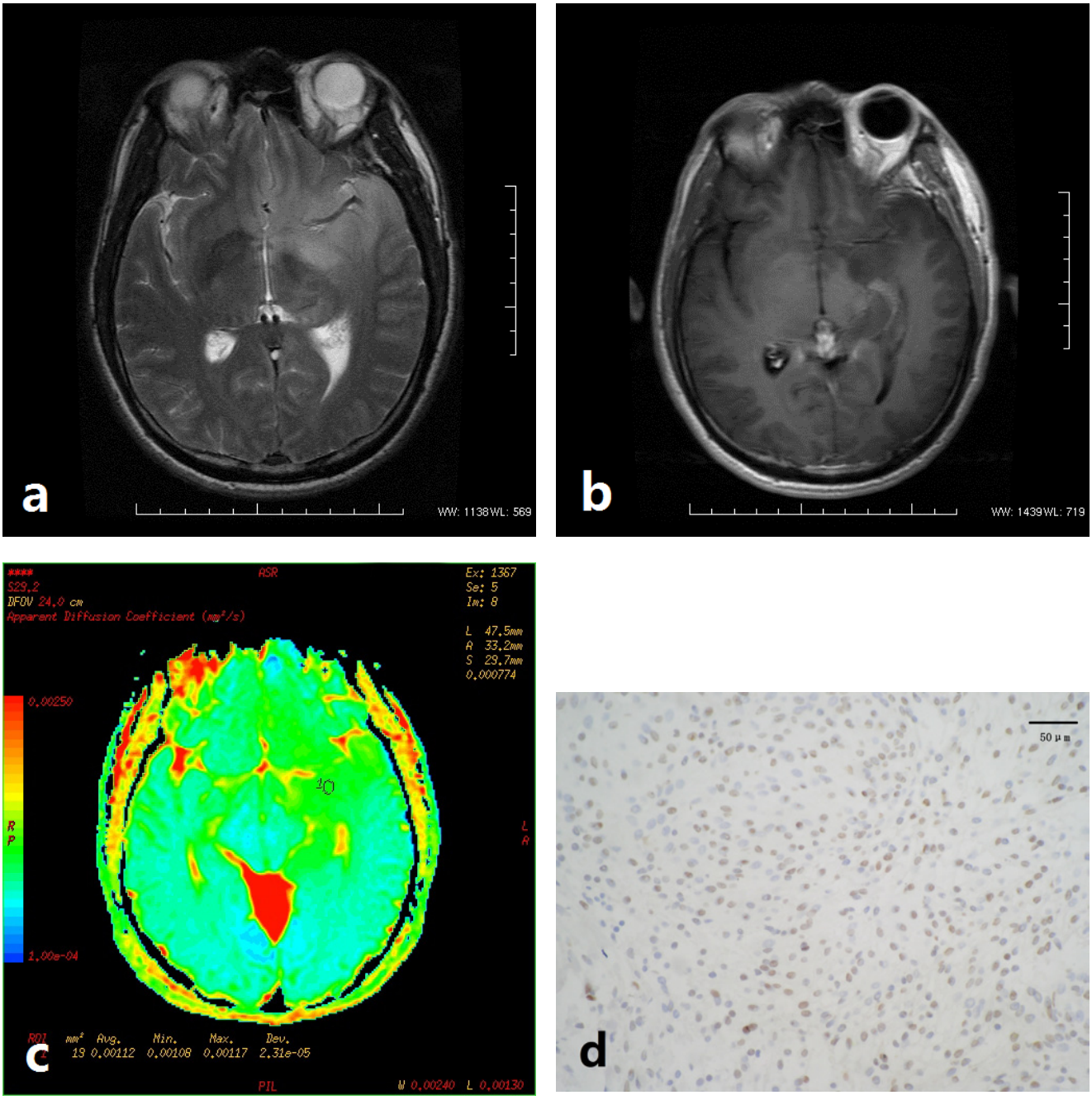

Figure 1. A 46-year-old male patient with grade II astrocytoma. a) T2-weighted MR image shows a slightly high signal intensity lesion in the left frontal and temporal lobe with unclear borderline and minor mass effect. b) Contrast enhanced T1-weighted MR image shows that enhancement of the lesion is subtle. c) ADC map with ROI placed in the solid part of the astrocytoma. d) Expression of PCNA; about 50% of nuclei were stained with light brown, which means a weak expression of PCNA. ADC, apparent diffusion coefficient; PCNA, proliferating cell nuclear antigen; ROI, region of interest. Scale bar = 50 µm.

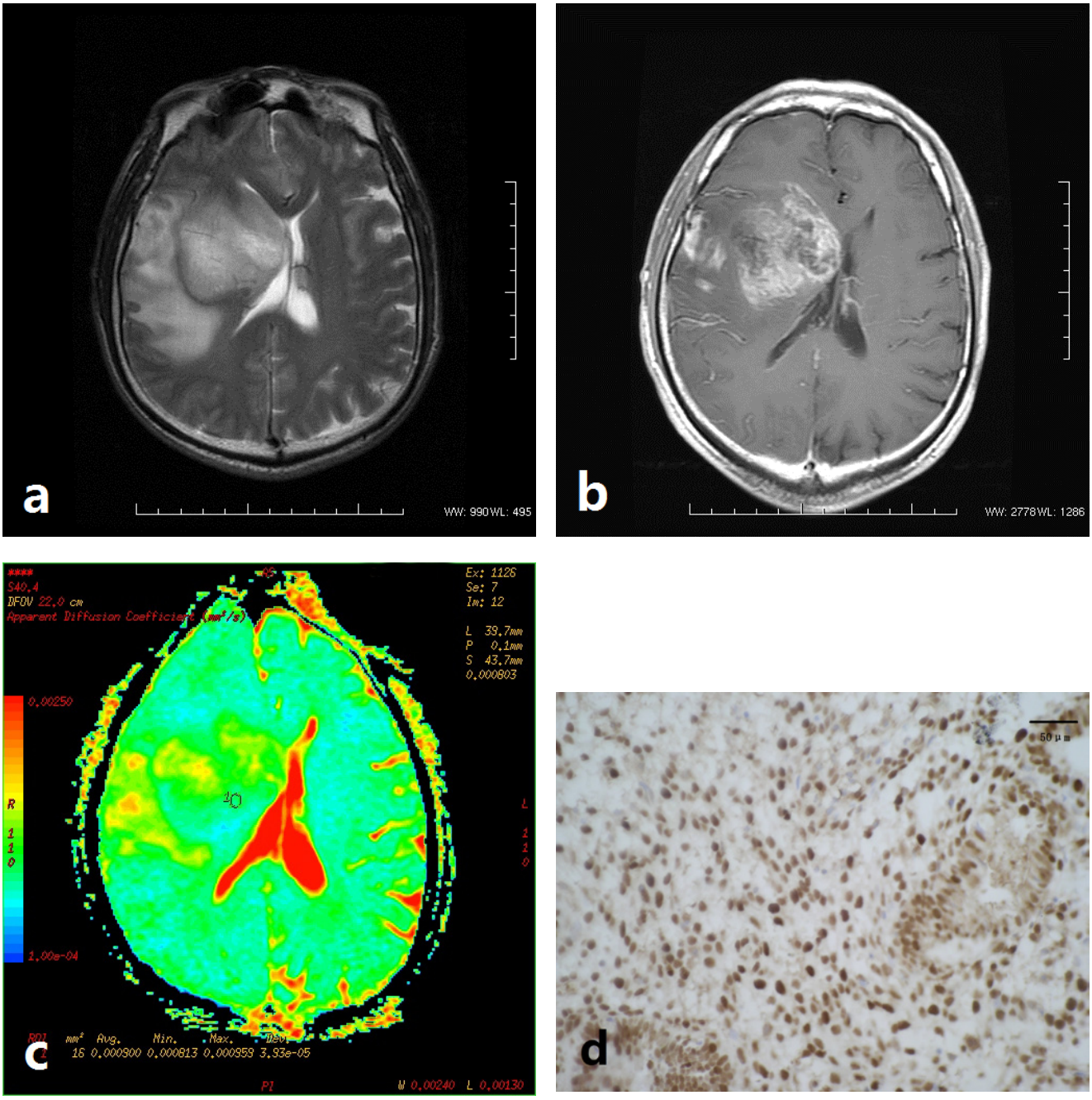

Figure 2. A 65-year-old male patient with glioblastoma. a) T2-weighted MR image shows a high signal intensity lesion in the right basal ganglia area and adjacent frontal and temporal lobe. The mass effect and peritumoral edema are significant. b) Contrast enhanced T1-weighted MR image shows the enhancement of the lesion is predominant and inhomogeneous. c) ADC map with a ROI placed in the solid part of the glioblastoma. d) Expression of PCNA, the cellular density is obviously higher than that of Fig. 1D. Most of the nuclei were stained with dark brown, which means a high expression of PCNA. ADC, apparent diffusion coefficient; PCNA, proliferating cell nuclear antigen; ROI, region of interest. Scale bar = 50 µm.

significant (t = -7.858, P = 0.000, Fig. 1D, Fig. 2D).

The average regional ADC were negatively correlated with the expression of PCNA significantly (r = -0.801, P = 0.000, Fig. 3).

Discussion

The expression of PCNA is high in the S, G2 and M stages of cell division, and low in the G0 and G1 stages. The degree of PCNA expression reflects cellular reproductive activity, which correlates significantly and positively with the pathological grade, the potential for recurrence,

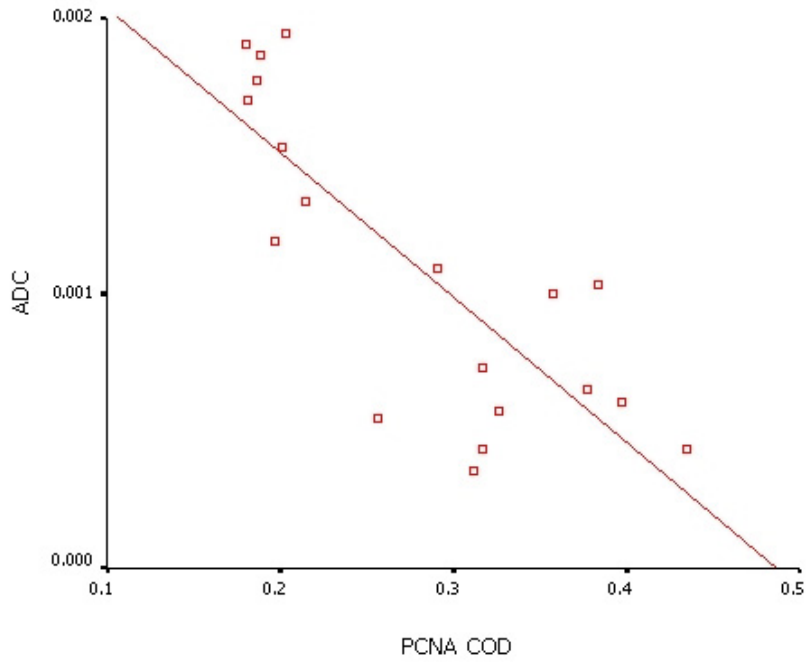

Figure 3. Scatterplot of PCNA and ADC shows the significant negative relationship between them (r = -0.801, P = 0.000). ADC, apparent diffusion coefficient; PCNA, proliferating cell nuclear antigen.

and survival time of malignant tumors [1, 2, 8]. Therefore, it is important to measure the degree of PCNA expression in evaluating the malignant potential and prognosis of tumors. In the present study there was also a tendency for the PCNA COD of astrocytomas to increase with an increase of the grade of the astrocytomas.

Tissue water diffusion is characterized by complexity. Its magnitude and direction depend on the permeability and spacing of diffusion barriers, viscosity of the suspending medium, and duration of diffusion observation [9]. Characterization is also complicated by bulk flow within capillaries and by tissue water active transport processes [10]. The protons in the brain included in the diffusion characterization are contained in water, whereas protons within macromolecules and membranes themselves make no contribution to this characterization because they are relatively immobile and have extremely short T2 values [11]. Therefore, white matter myelin plays a greater role in the diffusion direction and affects the ADC values. Although there are many determinants affecting ADC values in brain tissues, normal brain tissues are replaced by tumor cells in most affected areas of astrocytomas. Therefore, the water diffusion in astrocytomas may be mainly affected by tumor cellularity, because the motion of water in the interstitium is the main contributor to increased ADC values [11]. The proliferative activity of low grade astrocytomas is relatively lower than that of high grade astrocytomas. Low grade astrocytomas have low tumor cellularities and larger extracellular spaces compared with high grade astrocytomas. As a result, there are more formed elements per unit volume in high grade astrocytomas. Therefore, one can speculate that the differences in ADC values between low grade and high grade astrocytomas are related to differences in the volume and tortuosity of the extracellular space between these structures.

Based on the results of the present study, we found that the ADC values of astrocytomas decreased with an increase in tumor grade. But what is more important is the negative correlation between average regional ADC values and PCNA COD. We believe that the ADC values of the areas containing tumor cells are affected mainly by the size of the interstitium, as is observed in acute stroke, and that these values are accordingly related to tumor cellularity. Although cystic changes or necrosis within tumors potentially increase ADC values, it is not likely that the areas where the average regional ADC values were calculated included these areas, because the diffusion-weighted images had high spatial resolution (voxel size 1.72 x 1.72 x 1 mm), and thus cysts or necrosis within the tumor were easily differentiated from the solid portions. As is mentioned above, theoretically, ADC is mainly affected by cellularity. The negative correlation between average regional ADC and PCNA COD could be another objective piece of evidence, which indicates that the ADC value is a potential parameter to be used to evaluate the proliferative activity of astrocytomas.

There are some limitations in this study. One limitation is the study consisted of a small population. Only 19 patients were enrolled in this cohort. Another limitation is the concordance. To get the average regional ADC, we manually placed 5 to 10 30-40 mm^2 ROI within solid tumor components on the ADC maps. To measure the PCNA COD, ten 100 x magnification fields of each slice were selected randomly in one section. Therefore we could not guarantee the concordance of the tissue explored by the two methods although we chose the homogeneous solid part of tumor.

Although DWI is a non-invasive technique which could reveal the Brownian movement of water molecules of brain tissue, it has not been integrated into routine MR examination for evaluating brain tumors so far. One of the main reasons is most studies simply focus on comparing DWI with pathological grade. Little attention has been paid to do research using associated research achievements of protein, molecular or even genetic levels to evaluate DWI efficacy. There were only a few studies in recent years in this field. Tang *et al*. [12] found that ADC values correlated inversely with Ki-67 proliferation index and could help differentiate low-grade from aggressive meningiomas. Chen *et al*. [13] also found there was a negative correlation between the minimum ADC value and Ki-67 labeling index in neuroepithelial tumors. There are only three studies related to the correlation between ADC and PCNA expression up to now [14-16]. The ADC values were useful for the grading of gliomas. For the pathological grading system, many factors, such as cellularity and

vascularity, have been used as diagnostic criteria. Our results suggest not only the utility of the diffusion-weighted images but also the significance of tumor cellularity in establishing the grading of gliomas. Because the diffusion-weighted images depict the areas of greatest cellularity within the tumors, which may be the most active sites in gliomas, this technique may be useful in selecting the site of stereotactic biopsy.

In conclusion, the results of our study found significant negative correlations between average regional ADC and PCNA expression in humans, suggesting average regional ADC could be valuable to preoperatively grade astrocytomas.

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