

Original Article

Stretched-exponential model diffusion-weighted imaging as a potential imaging marker in preoperative grading and assessment of proliferative activity of gliomas

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Received March 2, 2018; Accepted July 8, 2018; Epub August 15, 2018; Published August 30, 2018

Abstract: Purpose: To assess the feasibility of using diffusion-weighted imaging (DWI) with the stretched-exponential model (SEM) for glioma grading and determining the correlations among parameters and proliferating cell nuclear antigen and Ki-67 expression. Materials and methods: Mono-exponential model-DWI (MEM-DWI) and SEM-DWI were performed in 104 patients with pathologically proven gliomas. The patients were divided into the training set ($n = 72$) and test set ($n = 32$). Apparent diffusion coefficient (ADC), solid tumor distributed diffusion coefficient (DDC), and whole tumor α values were measured. These parameters were applied as cut-off values to determine the predictive accuracy. Proliferating cell nuclear antigen and Ki-67 expression correlated with all parameters. Results: Significant differences between low-grade gliomas (LGG) and high-grade gliomas (HGG) were observed for all parameters ($P < 0.05$), and significant differences in the ability of DDC to distinguish between any two glioma grades ($P < 0.05$) were also evident. DDC showed the highest sensitivity and specificity for glioma grading and was negatively correlated with Ki-67 and proliferating cell nuclear antigen expression. DDC also showed greater predictive accuracy than ADC and α . Conclusion: SEM-DWI offers a better approach for glioma grading than MEM-DWI, and DDC may be a better imaging biomarker for grading and evaluating the proliferative activity of brain gliomas.

Keywords: Glioma, diffusion-weighted imaging, stretched-exponential model, distributed diffusion coefficient, pre-operative grading, proliferative activity

Introduction

According to the criteria of the 2007 World Health Organization (WHO) Classification of Tumors and their own biologic features, gliomas can be categorized into low-grade gliomas (LGG, WHO grades I and II), which are less aggressive and high-grade gliomas (HGG, WHO grades III and IV), which exhibit greater proliferative activity and aggression. As HGG have dismal prognoses and require postoperative chemoradiotherapy, unlike LGG, precise preoperative grading is very important for developing clinical strategies for these patients.

Currently, diffusion-weighted imaging (DWI) is widely applied for the characterization, preoperative grading and early determination of the-

rapeutic effectiveness in gliomas [1-11]. The DWI-derived apparent diffusion coefficient (ADC) has shown increasing potential as a non-invasive imaging biomarker for preoperative tumor grading and evaluating treatment response [1-6, 8-11]. Nevertheless, conventional mono-exponential model-DWI (MEM-DWI), which is based on the assumption that water diffusion behaves freely, is not accurate enough for glioma grading because evidence suggests the effectiveness of ADC varies greatly [12-15]. Water diffusion behavior in biological tissues may deviate from the Gaussian form, and the signal attenuation of brain water molecules typically does not fully exhibit mono-exponential decay. A non-Gaussian diffusion model may provide a more precise method for describing diffusion signal decay in vivo and reflect the

Table 1. Clinical characteristics of patients with brain gliomas

Characteristics	Training set	Test set
No. of patients	72	32
Age (y)*	43 (6-64)	45.5 (11-70)
Sex		
Female	29	13
Male	43	19
Pathological grading		
Grade II	34	14
Grade III	15	8
Grade IV	23	10

Data are represented as the median, with the range in parentheses. Age (y)* means the unit of age is year.

greater detail of biological patterns [16]. Therefore, advanced diffusion models have emerged, including the stretched-exponential model (SEM).

The assessment of glioma proliferative activity is important for predicting tumor behavior. Patients with the same histopathological tumor type receiving similar treatment may display diverse prognoses [17]. Ki-67 labeling index (LI) assays and proliferating cell nuclear antigen (PCNA), which are considered reliable immunohistochemical markers for the proliferation assessment, have been used to determine proliferative activity and predict the clinical outcome of gliomas [18-21].

Previous studies have demonstrated that the SEM-DWI derived α and distributed diffusion coefficient (DDC) values can be used to evaluate the average diffusion rate and intravoxel water diffusion in HGG and that SEM provides a more accurate estimate of the average diffusion rate than MEM [22-24], suggesting that SEM-DWI may be a better method for characterizing and evaluating brain tumors. However, these studies focused on only the characterization and evaluation of HGG, and differentiation between HGG and LGG. In our study, SEM-DWI was performed for glioma grading, and its diffusion parameters were further analyzed for correlations with proliferation activity, as measured by PCNA and the Ki-67 LI. Additionally, we compared SEM and conventional MEM.

Methods

Patients

The study was approved by the local Hospital Ethic Review Board, and informed consent was

obtained from all study subjects. One hundred four patients with histopathologically proven brain gliomas were enrolled from June 2010 to August 2014 in our hospital and were further divided into the training set ($n = 72$) and test set ($n = 32$) according to the time of MRI. Definition and validation of the cut-off values for DDC, ADC and α occurred in two phases. The first phase consisted of defining the cut-off values for DDC, ADC and α obtained at the optimal sensitivity and specificity for differentiating among different groups of gliomas based on the training set. The second phase consisted of a validation step in which the cut-off values were validated in the test set to prospectively predict the diagnostic test results (positive predictive value, negative predictive value, sensitivity, specificity and accuracy) of DDC, ADC and α and their ability to discriminate among different groups of gliomas. Detailed patient characteristics of the study population are described in **Table 1**. All patients underwent both MEM-DWI and SEM-DWI before any treatment was started. Biopsies of gliomas (grade II/III/IV, $n = 28/12/17$) were obtained from a proportion of the 72 cases in the training set to evaluate PCNA and Ki-67 expression.

Imaging protocol

All patients underwent an MRI scan on a 3.0 Tesla MRI system (Signa HDxt, GE Healthcare, Milwaukee, Wisconsin) with a 32-channel phased array head coil. The MRI protocols included axial T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), contrast-enhanced (CE) T1WI, and conventional MEM-DWI using b-values of 0 and 1000 s/mm². SEM-DWI using b-values of 0, 1000, 2000, and 3000 s/mm² was performed with a spin-echo echo-planar imaging sequence and the following parameters: 20 axial slices, repetition time (TR), 3000 ms; echo time (TE), 17.2 ms; slice thickness, 5 mm; spacing, 1.5 mm; field of view (FOV), 24 × 24 cm²; matrix, 128 × 128; NEX, 2; flip angle (FA), 90°; and number of signal averages, 1 for b-value of 0 and 1000 s/mm² and 3 for b-values of 2000 and 3000 s/mm² (to reduce noise at higher b-values). The entire process took 4 min 57 s to complete.

Data processing

Data postprocessing was performed on a GE 4.4 workstation by using the MADC program for both MEM-DWI and SEM-DWI analysis.

Table 2. Statistical analyses of DDC, α and ADC and their differentiation of LGG from HGG and discrimination among grade II, III and IV gliomas

Group	DDC (10^{-3} mm ² /s)	ADC (10^{-3} mm ² /s)	α
LGG	1.693 $\pm$ 0.630	1.433 $\pm$ 0.433	0.713 $\pm$ 0.060
HGG	0.832 $\pm$ 0.300 [†]	0.869 $\pm$ 0.207 [†]	0.667 $\pm$ 0.051 [†]
Grade II	1.693 $\pm$ 0.630	1.433 $\pm$ 0.433	0.713 $\pm$ 0.060
Grade III	1.081 $\pm$ 0.324 [*]	1.000 $\pm$ 0.242 [*]	0.665 $\pm$ 0.044 [*]
Grade IV	0.670 $\pm$ 0.123 ^{*Δ}	0.784 $\pm$ 0.126 [*]	0.669 $\pm$ 0.056 [*]
NAWM	0.810 $\pm$ 0.191	0.809 $\pm$ 0.137	0.729 $\pm$ 0.057

Notes: Data are presented as the means $\pm$ standard deviations. [†]Statistically significant difference between LGG and HGG; NAWM, normal-appearing white matter; ^{*}Statistically significant difference between grade II and higher-grade (grade III and IV) gliomas; ^ΔStatistically significant difference between grade III and IV gliomas.

The conventional ADC was calculated by using the following MEM formula:

$$S_b = S_0 \exp(-b \times ADC) \quad (1)$$

where S_0 and S_b are the signal intensities obtained with the b_0 and b_1 values (0 for b_0 and 1000 for b_1 , units are s/mm²), which are the most commonly used in brain.

The parameters DDC and α were calculated by fitting SEM to the DWI data using the formula as follows:

$$S_b = S_0 \exp\{-(b \times DDC)^\alpha\} \quad (2)$$

where DDC represents the mean intravoxel diffusion rate and has the same properties and units as ADC, and α is the intravoxel water molecular diffusion heterogeneity index, a parameter ranging from 0 to 1. DDC and α maps are generated after fitting the SEM to the obtained DWI data using 4 b values (0, 1000, 2000, 3000 s/mm²), thereafter permitting the quantitative evaluation of α and DDC values.

ADC and DDC values were measured three times by placing freehand-drawn regions of interest (ROI) (range: 32-57 mm²) on the solid portions of tumors. Areas with cysts, necrosis, calcification and hemorrhage were avoided. However, when measuring α values, we included the whole tumor in the ROI for the sake of evaluating tumor heterogeneity. ADC, DDC and α were also measured on the contralateral normal-appearing white matter (NAWM). Standard MRI, including T2WI and CE-T1WI, was used to cross-reference solid portions of the tumor to

the ADC, DDC and α maps. The ROIs were drawn on the corresponding T2-FLAIR or CE T1-FLAIR images and then copied to the ADC and DDC maps on the GE 4.4 workstation by using the MADC program. Two board-certified radiologists (with over 6 years and 20 years of experience, individually) blinded to the patients' clinical and pathologic data selected and confirmed the ROIs independently.

PCNA and Ki-67 immunohistochemical staining and analysis

Streptomycin avidin-biotin-peroxidase complex was used on 4- μ m-thick, formalin-fixed and paraffin-embedded brain tumor excisional biopsies to evaluate PCNA and Ki-67 protein expression using monoclonal murine antibodies. Positive detection of PCNA and Ki-67 appeared as a brown nuclear stain with a diffuse pattern. Quantitative estimation based on the percentage of positive cells in the highest-density stained areas was performed by two experienced neuropathologists. A total of 1,000 cells, excluding inflammatory and vessel cells, were counted in ten high-power fields, and the percentage of positive-stained cells was recorded as the PCNA or Ki-67 LI. Any discrepancies between the evaluations of the two neuropathologists were solved by consensus.

Statistical analysis

One-way ANOVA was used to compare the differences in ADC, DDC and α values of the grade-II, -III and -IV glioma groups. To compare ADC and α values between LGG and HGG, the independent samples t-test was used. Pearson's correlation coefficient was employed to evaluate the correlations between tumor ADC and DDC values and between the Ki-67 LI (or PCNA LI) and DDC (or ADC and α). A Spearman rank correlation coefficient was calculated to evaluate the correlation between the Ki-67 LI (or PCNA LI) and the tumor grade. A receiver operating characteristic (ROC) curve was used to assess the diagnostic sensitivity and specificity of ADC, DDC, and α in glioma differentiation. Statistical analyses were conducted using SPSS software for Windows (version 17.0, SPSS, Chicago, Ill); in all cases, $P < 0.05$ was considered statistically significant.

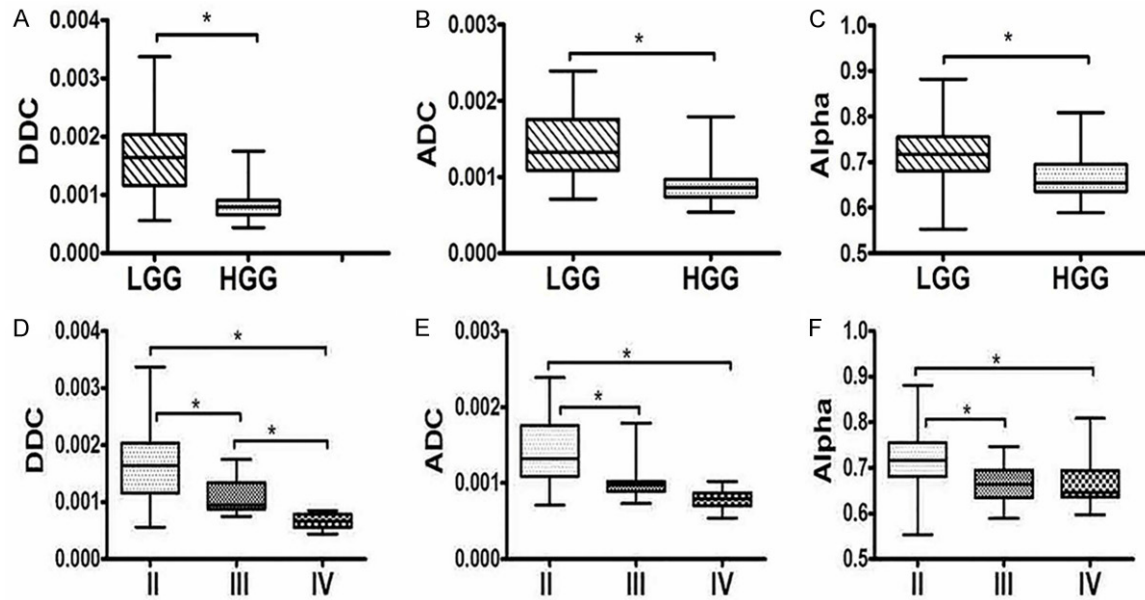


Figure 1. Box plots of DDC, ADC and α (alpha) values discriminating between LGG and HGG (A-C) and between grade II, III and IV gliomas (D-F). DDC (A), ADC (B) and α (C) values of LGG were significantly higher than those of HGG; DDC (D), ADC (E) and α (F) values of grade II were significantly higher than those of grade III or IV; DDC (D) values of grade III were significantly higher than those of grade IV. *, statistically significant difference ($P < 0.05$).

Results

Training set results

Comparison of MEM-DWI and SEM-DWI for glioma grading: Comparisons of ADC, DDC and α between LGG and HGG showed that the parameter values of LGG were significantly higher than those of HGG (ADC: 1.433 ± 0.433 for LGG vs. 0.869 ± 0.207 for HGG, $P = 0.000$; DDC: 1.693 ± 0.630 for LGG vs. 0.832 ± 0.300 for HGG, $P = 0.000$; α : 0.713 ± 0.060 for LGG vs. 0.667 ± 0.051 for HGG, $P = 0.001$). Comparisons of ADC, DDC and α between different glioma grades showed that the parameter values of grade II were significantly higher than those of grades III or IV (ADC: 1.433 ± 0.433 for grade II vs. 1.000 ± 0.242 for III or 0.784 ± 0.126 for IV, all $P = 0.000$; DDC: 1.693 ± 0.630 for grade II vs. 1.081 ± 0.324 for III or 0.670 ± 0.123 for IV, all $P = 0.000$; α : 0.713 ± 0.060 for grade II vs. 0.665 ± 0.044 for III, $P = 0.007$, or 0.669 ± 0.056 for IV, $P = 0.005$). Comparing ADC, DDC and α between grade III and IV showed that only DDC was significantly different (DDC: 1.081 ± 0.324 for grade III vs. 0.670 ± 0.123 for IV, $P = 0.01$; ADC: 1.000 ± 0.242 for grade III vs. 0.784 ± 0.126 for IV, $P = 0.052$; α : 0.665 ± 0.044 for grade III vs. 0.669 ± 0.056 for IV, $P = 0.827$). The values of ADC, DDC and α

were significantly different between tumor and the contralateral NAWM ($P = 0.000$). (units of ADC, DDC: $10^{-3} \text{ mm}^2/\text{s}$, range of α : 0-1. **Table 2; Figure 1**). Representative examples of the ADC, DDC and α maps of grade II, III, and IV gliomas are shown in **Figure 2**.

ROC curve analysis of MEM-DWI and SEM-DWI to grade gliomas: For differentiating HGG from LGG, DDC and ADC values showed the same area under the curve (AUC), which was higher than that of α (0.918, 0.918 and 0.743, respectively), while DDC had the highest sensitivity among the three parameters (94.1%, 85.3%, and 64.7%, respectively). All three parameters displayed high specificity (89.5%, 94.7% and 81.6%, respectively) (**Table 3**). The corresponding ROC curves are shown in **Figure 3A**. In discriminating between grade II and III gliomas and between grade III and IV gliomas, DDC showed high AUC values (0.836 and 0.968, respectively) and sensitivity (94.1% and 93.3%, respectively) in solid tumors; DDC specificity values were 73.3% and 91.3%, respectively (**Table 3; Figure 3B, 3C**). Because ADC and α values did not differ significantly between grades III and IV, we did not conduct further ROC analysis for this comparison.

Correlation analysis between DWI (MEM-DWI and SEM-DWI) and proliferative activity (Ki-67

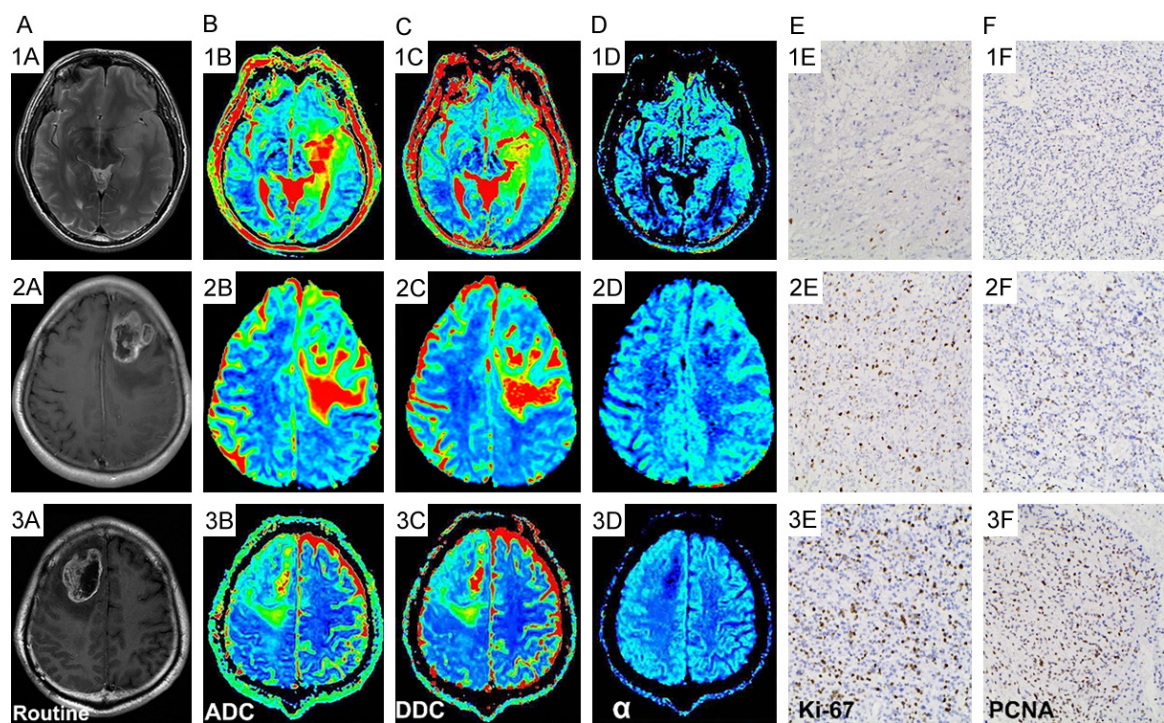


Figure 2. Correlation of ADC, DDC and α (Alpha) with glioma grades, Ki67 and PCNA. Rows 1-3 correspond to a 51-year-old male with a grade II glioma in the left hippocampus, a 58-year-old female with a grade III glioma in the left frontal lobe and a 51-year-old male with a grade IV glioma in the right frontal lobe. Columns A-F are routine reference images, ADC, DDC, α , Ki-67 and PCNA images. Routine images (1A, 2A and 3A) are the T2 weighted image for the grade II glioma (1A) and contrast-enhanced T1 weighted images for the grade III (2A) and grade IV (3A) gliomas. ADC (1B, 2B and 3B), DDC (1C, 2C and 3C), α (1D, 2D and 3D) of solid tumors showed that signal intensity decreased as tumor grade increased, while Positive Ki-67 (1E, 2E and 3E) and PCNA (1F, 2F and 3F) staining showed that the Ki-67 LI and PCNA LI increased as tumor grade increased (Ki-67, 2%, 40% and 70%; PCNA, 1%, 45% and 75%, respectively).

Table 3. ROC curve analysis of DDC, ADC and α values in glioma grading

Parameters	AUC	Cut-off value (10^{-3} mm ² /s)	Sensitivity (%)	Specificity (%)
*HGG vs. LGG				
DDC	0.918	1.065	94.1	89.5
ADC	0.918	1.022	85.3	94.7
α	0.743	0.698	64.7	81.6
†Grades II and III				
DDC	0.836	1.065	94.1	73.3
Grades III and IV				
DDC	0.968	0.811	93.3	91.3

*ROC curve analysis of DDC, ADC and α values and their differentiation of HGG from LGG. †ROC curve analysis of DDC and its differentiation between grade II and III and between grade III and IV gliomas. AUC, area under the curve.

and PCNA): A strong positive correlation was observed between DDC and ADC in the solid parts of tumors ($R = 0.872$, $P = 0.000$), as shown in **Figure 4F**. Correlation analyses bet-

ween PCNA LI or Ki-67 LI and increasing glioma grade found significant positive correlation ($R = 0.550$, $P = 0.000$ and $R = 0.622$, $P = 0.000$, respectively). Furthermore, a strong correlation was observed between PCNA LI and Ki-67 LI ($R = 0.540$, $P = 0.000$). An analysis of the correlation between DWI parameters and proliferative activity revealed statistical correlations between each DWI parameters (ADC, DDC and α) and PCNA LI ($R = -0.304$, $P = 0.021$; $R = -0.305$, $P = 0.021$; and $R = -0.305$, $P = 0.021$, respectively) (**Figure 4A, 4C, 4D**). However, negative correlations were observed between DDC values and Ki-67 LI and between ADC values and Ki-67 LI ($R = -0.363$, $P = 0.006$ and $R = -0.343$, $P = 0.009$, respectively) (**Figure 4B, 4E**), while no statistical correlation was observed between α and Ki-67 LI values ($R = -0.236$, $P = 0.077$).

SEM-DWI for glioma grading and proliferative activity assessment

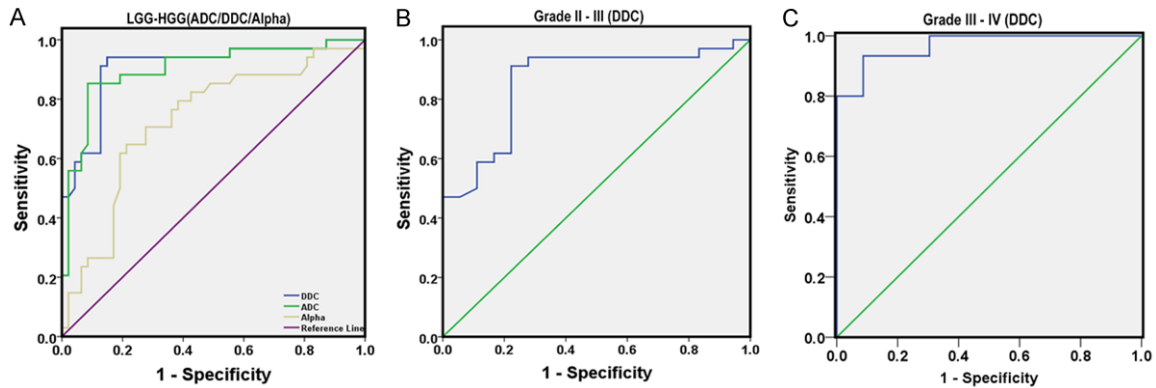


Figure 3. ROC curves for DDC, α and ADC to discriminate different glioma grades. Between LGG and HGG (A), DDC and ADC (area under the curve (AUC), 0.918 and 0.918, respectively) showed better discrimination effects than α (AUC, 0.743). ROC curves for the DDC in differentiating between grades II and III (B) and between grades III and IV (C) showed both high AUC value (0.836, 0.968).

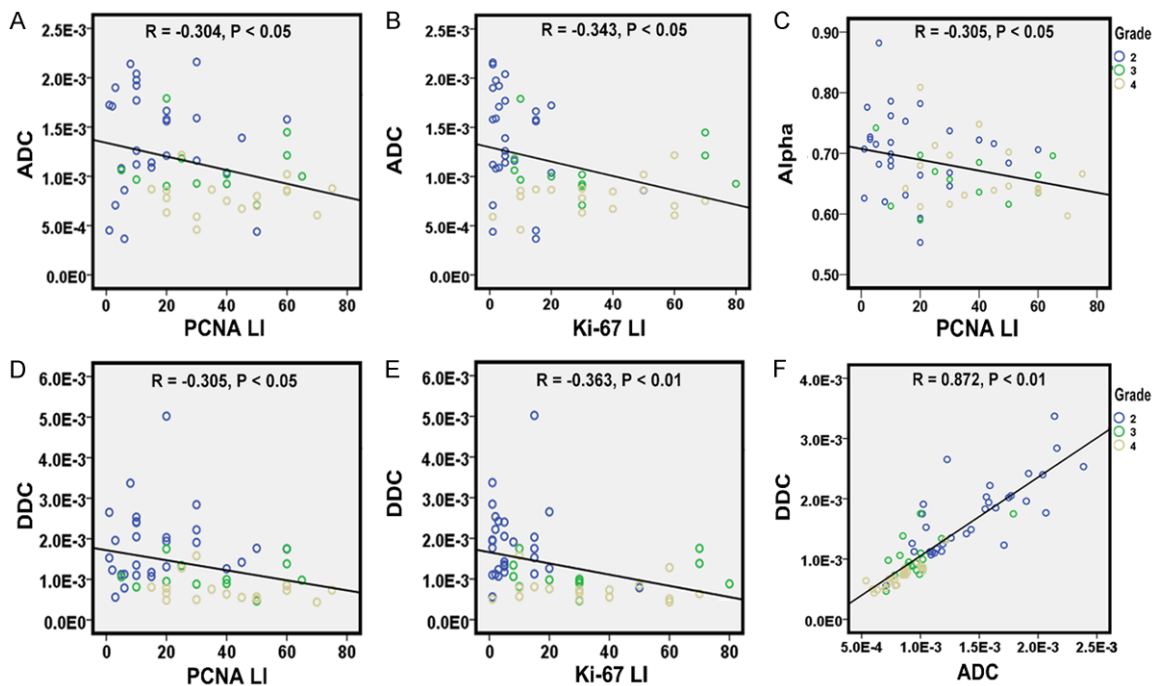


Figure 4. Scatter diagram showing statistically low correlations between ADC and the PCNA LI ($R = -0.304$, $P < 0.05$) (A), between ADC and the Ki-67 LI ($R = -0.343$, $P < 0.05$) (B), between α (alpha) and the PCNA LI ($R = -0.305$, $P < 0.05$) (C), between DDC and the PCNA LI ($R = -0.305$, $P < 0.05$) (D) and between DDC and the Ki-67 LI ($R = -0.363$, $P < 0.01$) (E). With increasing tumor grade, the Ki-67 LI increased, whereas DDC, ADC and α values decreased. The scatter diagram also shows a strong positive correlation between DDC and ADC values in the solid portions of tumors ($R = 0.872$, $P < 0.01$) (F).

Test set results

Diagnostic test for MEM-DWI and SEM-DWI in glioma grading: Using the cut-off values derived from the training set, the results obtained from the diagnostic test for differentiating HGG and LGG using DDC, ADC and α values are listed in Table 4. In general, the DDC had a much higher

positive predictive value, negative predictive value, sensitivity and diagnostic accuracy than the ADC or α (positive predictive value and negative predictive value: 94.1% and 86.7%, respectively, for DDC; 93.8% and 81.2%, respectively, for ADC; and 76.5% and 66.7%, respectively, for α . sensitivity and specificity: 88.9% and 92.9%, respectively, for DDC; 72.2% and

Table 4. Diagnostic test assessing the ability of DDC, ADC and α values to distinguish gliomas in the test set

	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
DDC					
LGG and HGG	94.1	86.7	88.9	92.9	90.6
II and III	87.5	92.9	87.5	92.9	90.9
III and IV	90.0	87.5	90.0	87.5	88.9
ADC					
LGG and HGG	93.8	81.2	72.2	92.9	87.5
α					
LGG and HGG	76.5	66.7	72.2	71.4	71.9

Note: PPV = positive predictive value, NPV = negative predictive value.

92.9%, respectively, for ADC; and 72.2% and 71.4%, respectively, for α . diagnostic accuracy: 90.6%, 87.5%, and 71.9% for DDC, ADC, and α , respectively). Moreover, the DDC had high positive predictive value, negative predictive value, sensitivity, specificity and diagnostic accuracy in discriminating between grade II and III gliomas and between grade III and IV gliomas (positive predictive value and negative predictive value: 87.5% and 92.9%, respectively, for II vs. III and 90.0% and 87.5%, respectively, for III vs. IV; sensitivity and specificity: 87.5% and 92.9%, respectively, for II vs. III and 90.0% and 87.5%, respectively, for III vs. IV; and diagnostic accuracy: 90.9% and 88.9%, for II vs. III and III vs. IV, respectively) (Table 4).

Discussion

The principal finding of our study is that SEM-DWI better distinguishes different grades of gliomas preoperatively and its derived DDC correlates more significantly with PCNA and Ki-67 LI than the ADC derived from MEM-DWI. We focused on the utility of SEM-DWI for the preoperative differentiation of different grades of brain gliomas, as grades II and III or grades III and IV are usually not distinguishable with standard MRI sequences. We also evaluated the correlations between DWI parameters and glioma proliferative activity (using PCNA and Ki-67 staining).

Currently, imaging is no longer used simply to evaluate changes in size or enhancement patterns. Improved MRI techniques have shown significant potential for the evaluation of key pathological features of gliomas, including cellularity, mitotic activity, angiogenesis, invasive-

ness, and necrosis, further extending the knowledge available for preoperative glioma grading and post-treatment evaluation [25]. Conventional MEM-DWI derived ADC has been considered an important clinical biomarker for predicting prognosis and histologic grade. In the current study, we found significantly higher ADC values in LGG than in HGG. Statistical analysis showed that there were statistically significant differences in ADC differences existed between grade II gliomas and grade III or IV gliomas. However, no significant difference was observed between grade III and IV

gliomas. Therefore, ADC values only differentiate HGG from LGG, as described in previous studies [1, 5, 6]. Many factors, such as intracellular high viscosity, macromolecular crowding, restriction effects, cell membrane and extracellular tortuosity effects, are considered having contribution to water diffusion behavior in vivo, and an ADC value calculated from a simple MEM model cannot contain all information about diffusion [16].

Hall's study demonstrated that SEM was a more suitable model for describing diffusion-weighted signal decay caused by the heterogeneous environment of the spins [26]. Additionally, SEM-derived parameters have been shown to be more reliable and reproducible [27]. Through equations (1) and (2), the model of diffusion-weighted signal decay is equal to MEM when the α value approaches 1, i.e., at high intravoxel diffusion homogeneity. Conversely, higher intravoxel diffusion heterogeneity and a lower α value result in a high complex of diffusion-weighted signal decay presenting as multi-exponential decay [28-30].

Our study findings were encouraging with respect to differentiation among different grades of gliomas. Although the AUC of the DDC in discriminating between LGG and HGG was the same as that of the ADC, the sensitivity of the DDC was higher than that of the ADC. In addition, DDC values significantly differed between any two groups among grade II, III and IV gliomas; specifically, DDC values decreased significantly with increasing tumor grade. Thus, the DDC not only differentiates LGG and HGG with high sensitivity but also distinguishes grade IV from grade III gliomas, whereas the ADC may

not. We noted a highly positive correlation between DDC and ADC values in the solid parts of gliomas. Cellularity contributed to these findings, as the measured ADC value within a tumor correlates well with areas of high cellularity and a higher grade [3, 31], confirming the previously known fact that ADC value correlates with cellularity. Diffusion in biological tissue has traditionally been quantified using an MEM, i.e., using the ADC. However, it is widely known that a single glioma, especially if it is of high grade, may demonstrate a wide spectrum of histological features that range from grade II to grade IV (high heterogeneity) within the same tumor, resulting in diffusion complexity. Therefore, conventional DWI is not accurate enough to describe the diffusion characteristics of gliomas, especially in HGG [25], as demonstrated by our study and in previous studies by others [22, 28-30]; however, the DDC displays sufficient accuracy. The DDC may possess greater accuracy because it has the same properties and units as the standard diffusion coefficient and that it can be thought of as a composite of individual ADCs weighted by the volume fraction of water in each part of the continuous distribution of ADCs [22, 24, 28-30]; the DDC may therefore provide more a complete and accurate depiction of tissue water diffusion in the presence of non-mono-exponential decay.

The α -index, another parameter derived from the SEM, provides a new type of image contrast (different from the MEM), related to the degree of intravoxel water diffusion heterogeneity [22, 24, 28-30]. In the present study, we found that α values distinguished HGG from LGG but were unable to differentiate grade IV from grade III gliomas. This result may be explained by the fact that HGG has greater heterogeneity than LGG and that grade III and IV gliomas have similar pathological characteristics with similar degrees of heterogeneity [25, 32, 33]. Gliomas, particularly glioblastoma multiforme, are associated with considerable histological heterogeneity (densely cellular and pleomorphic tumors with highly mitotic activity, endothelial proliferation, and necrosis) [34, 35]. High heterogeneity may lead to the presence of many different compartments with different proton pools (lower α values) in gliomas. Another finding in our study was that the α value of a tumor was significantly lower and its standard deviation was slightly higher than that of NAWM due to the

heterogeneity of gliomas, which aligns with the findings of prior studies [24, 29]. In this way, α values may be used to perform diffusion heterogeneity imaging of gliomas.

The ability of DDC, ADC and α to differentiate HGG from LGG and for DDC to discriminate grades II and III and grades III and IV in the test set validated the findings from the training set, showing that DDC values are more sensitive than ADC values for distinguishing HGG from LGG and that DDC values have strong discriminatory power for differentiating between grade II and III and grade III and IV gliomas.

When we assessed the correlations among calculated parameters and PCNA and Ki-67 expression, we obtained the following results. First, a significantly positive correlation was observed between PCNA LI or Ki-67 LI and increasing grade. Specifically, PCNA and Ki-67 LI increased significantly with increasing malignancy grade, as demonstrated in studies by Torp SH and Chaloob MK [18, 36]. Additionally, a statistically negative correlation was noted between DDC or ADC values and PCNA LI, as well as between DDC or ADC values and Ki-67 LI, suggesting that DDC and ADC values decrease with increasing tumor grade and PCNA or Ki-67 LI. Lastly, there was a statistically significant correlation between α and PCNA LI, but no statistical correlation was observed between α and Ki-67 LI values ($R = -0.236$, $P > 0.05$). All these data suggested that DDC and ADC values are potential alternative to PCNA and Ki-67 LI for predicting proliferative activity in gliomas, as the measurement of DDC and ADC values is noninvasive. Based on its higher correlation with PCNA and Ki-67 LI, DDC seems to be the better choice.

There are several limitations of our study. First, the patient population did not include patients with grade I gliomas due to the nature of our patient population composition. Second, the regions used to measure DDC, ADC and α did not exactly correspond to the regions obtained for Ki-67 LI assessment via surgery or biopsy samples, although we requested that the neurosurgeons obtain the corresponding parts of the tumors.

In conclusion, SEM-DWI provides a more sensitive and accurate estimate for the preoperative grading of gliomas than conventional MEM-

DWI. DDC is a potential imaging biomarker to differentiate glioma grade preoperatively and predicts the proliferative activity of gliomas in a more feasible manner. Additionally, α could be used as an imaging biomarker of glioma heterogeneity.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (no 30870702, no 81570462, and no 8117-1308). The authors thank all the participants for their contributions to this project.

Disclosure of conflict of interest

None.

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