Original Article
MinADC values predict prognosis in patients with low-grade and high-grade gliomas by 3.0-T MRI

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Abstract: Aim: To retrospectively evaluate the minimum apparent diffusion coefficient (minADC) value for predicting the malignancy and prognosis of glioma. Materials and methods: A total of 9 low-grade glioma (LGG) patients with low Ki-67 labeling index (LI), 7 low-grade glioma patients with a high Ki-67 LI and 22 high-grade glioma (HGG) patients underwent diffusion-weighted (DW) imaging with 3.0-T magnetic resonance (MR) and head coil. The patients were followed-up for 1-2.5 years. The minADC glioma value was calculated from two layers of DW images (b=0, 1000 s/mm^2). Immunohistochemical Ki-67 staining was used to determine the glioma Ki-67 LI. Results: Progression-free survival (PFS) was 100% in LGGs with a low Ki-67 LI, 42.9% in LGGs with a high Ki-67 LI and 35.0% in HGGs. The minADC glioma value was negatively correlated with the Ki-67 LI (r=-0.688, P=0.000). An inverse association between these parameters was also noted in LGGs (r=-0.529, P=0.035) and HGGs (r=-0.483, P=0.023). The mean minADC values (10^{-3} mm^2/s) of LGGs with high Ki-67 LIs and low Ki-67 LIs were (0.76±0.18) and (1.12±0.32), respectively. The former was significantly lower than the latter (P=0.008). The minADC values of LGGs with high Ki-67 LIs and HGGs were (0.76±0.18) and (0.69±0.25), respectively. This difference was not significant (P=0.559). Conclusion: Our results indicate that the minADC value can predict the malignancy and prognosis of glioma.

Keywords: Glioma, magnetic resonance, DWI, minADC, Ki-67

Introduction

Glioma is the most common type of primary brain tumor and a critical cause of cancer mortality. The World Health Organization (WHO) [1] notes that gliomas are classified into 4 grades based on prognosis severity. However, the prognosis of these tumors in the clinical setting varies despite having the same histopathologic grade and equivalent treatments. Some reports [2-5] suggest that some low-grade gliomas (LGGs) have anaplastic transformation characteristics with poor prognosis. The current WHO glioma classification is limited in its value to predict clinical outcome and survival. Additional prognostic markers are required. Many studies [3, 6-15] have focused on the clinical value of the proliferative activity in these tumors, especially the Ki-67 labeling index (LI).

The Ki-67 LI is a core antigen that is present in proliferating cells and absent in quiescent cells. This antigen is expressed in all cycling cells except resting cells in the G0 phase. Many studies [6, 7, 9, 10, 12-14] have determined the prognostic value of Ki-67 for survival in glioma patients. Chen W's [6] meta-analysis found a significant association between Ki-67 overexpression and worsening prognoses in patients with glioma. Preusser *et al.* [14] found that the Ki-67 index remained an independent prognostic factor. The prognostic value of Ki-67 as a marker of survival has been advised in many reports. Ki67/MIB-1 proliferation indexes in diffuse LGGs are generally low, with values mostly below 4, 5, and 6% in diffuse astrocytoma, oligodendrogliomas and oligoastrocytomas, respectively [8, 9, 13, 16]. However, Ki-67 LI was only obtained after surgery. A method enabling preoperative tumor prognosis assessment will be very useful for therapy.

Magnetic resonance (MR) diffusion-weighted (DW) imaging has recently been proposed as a means to provide noninvasive, spatially specific information about tumor cellularity [17-19]. DWI

Table 1. Pathologic diagnosis of gliomas in our study

Histologic group	Number of cases
(1) Low grade gliomas (LGGs)	
Astrocytoma	8
Oligodendroglioma	7
Oligoastrocytoma	1
(2) High grade gliomas (HGGs)	
Astrocytoma	1
Anaplastic astrocytoma	2
Oligodendroglioma	2
Anaplastic oligodendroglioma	4
Anaplastic mixed glioma	1
Glioblastoma	12

reflects water molecule mobility in vivo by the apparent diffusion coefficient (ADC) value. The ADC value has been used to preoperatively determine the glioma grade. However, substantial overlap in the regional ADC values between gliomas of different grades was present [20, 21]. Few studies [22, 23] have examined correlations between minADC values and Ki-67 LI. Diffusion-weighted imaging has been proposed as a candidate marker for survival prediction in high grade glioma (HGG) patients [23, 25]. However, using the minADC value to predict the prognosis of gliomas, including LGGs, is controversial. Therefore, the purpose of this study was to investigate the prognostic implication and preoperatively assess malignancy of gliomas by minADC value.

Material and methods

Patients

From May 2012 to September 2014, 38 patients with a pathologic diagnosis of gliomas underwent MRI. Their median age was 46 years (range, 13-68 years), and there were 27 males and 11 females. Sixteen patients met the WHO criteria for LGG (8 with astrocytoma, 7 with oligodendroglioma, and 1 with oligoastrocytoma), 22 patients were HGGs (1 with astrocytoma, 2 with anaplastic astrocytoma, 2 with oligodendroglioma, 4 with anaplastic oligodendroglioma, 1 with anaplastic mixed glioma, and 12 with glioblastoma) (**Table 1**).

The patients met the following criteria: (1) diagnosed with astrocytoma tumors, oligodendroglioma tumors or mixed gliomas by histopathol-

ogy according to the WHO criteria; (2) had Ki-67 records in electronic medical records; (3) underwent a 3.0-T MRI examination that included conventional MR images and DW images before surgery. The exclusion criteria were the following: (1) DW images that were unable to show the lesions because of the small volume; (2) tumors with too many cystic, necrotic, or hemorrhagic components to permit the placement of regions of interest on solid components; and (3) having undergone stereotactic biopsy, radiation and chemotherapy before MRI. The time interval between MRI and surgery ranged from 1 to 20 days (median, 3 days). A one-year follow-up was performed after the initial treatment.

The Ethical Committee of Provincial Hospital Affiliated with Shandong University approved this retrospective study and waived the need for informed consent.

MR imaging

MR examinations were performed on a 3.0-T MR imaging system (MagnetomVerio, Siemens, Erlangen, Germany) with an eight-channel phased-array head coil. Conventional MR and DW images were acquired during the same imaging session without repositioning. Conventional MR imaging included an axial T1-weighted sequence, axial fast spin-echo T2-weighted, axial fluid-attenuated inversion recovery (FLAIR), and triplanar contrast enhanced T1-weighted sequence. The contrast agent was gadopentetatedimeglumine (Beilu, Beijing, China).

DW imaging was performed with a spin shot echo-planar imaging sequence (repetition time/echo time in ms, 6600/100 ms; number of signals acquired, one; section thickness, 6.5 mm; intersection gap, 2 mm; 20 sections; matrix, 200×200; field of view, 220×220 mm) with three orthogonal directional motion-probing gradients (b=1000 sec/mm^2), followed by automatic generation of isotropic DW images. Images without motion-probing gradients (b=0 sec/mm^2) were also simultaneously obtained.

Data processing

ADC maps were automatically generated on a Syngo workstation (SIEMENS, Germany). The ADC value was calculated with the following formula: *ADC*=-In (s (*b*=1000)/s (*b*=0))/1000,
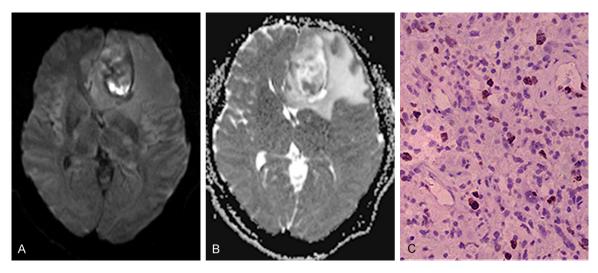
Figure 1. A 51-year-old patient with Glioblastoma, WHO 4 grade. A: High-grade glioma (HGG) was on hyperintense on diffusion-weighted image (b=1000 s/mm^2), B: Hypointense on apparent diffusion coefficient map (b=0, 1000 s/mm^2), C: Ki-67 staining of Glioblastoma (×400), Ki-67 LI: 20%.

where S (b=1000) and S (b=0) are the signal intensities of images with and without DW, respectively.

A neuroradiologist with more than 10 years of experience with head MRI reviewed the conventional MR with and without contrast-enhanced and DW images. This individual then measured the ADC values on the solid tumor components. The neuroradiologist was blind to the pathology and Ki-67 LI of each sample. We first selected all continuous sections, including the tumors. We then carefully placed the regions of interest (area, approximately 0.1 cm^2, 10 pixels) on non-overlapping regions of each selected section of solid tumor components, except the first and last sections. The regions were placed one by one, avoiding cystic, necrotic, or hemorrhagic tumor components. The ADC values were recorded, and we chose the lowest value as the minimum.

Pathological analysis

Neurosurgeons performed surgical removal based on MR imaging information, tumor location, and the patients' performance status. All tumors were graded according to the 2007 WHO criteria. Specimens were obtained from enhanced and non-enhanced areas of each tumor. The Ki-67 LI was retrospectively determined in 38 patients in addition to a conventional histopathologic evaluation.

Immunohistochemical staining was performed by a neuropathologist with Ki-67 mouse mono-

clonal antibody (MIB-1, ZSGBBIO, Beijing, China). The neuropathologist with 10 years rich experience was blind to the ADC values and glioma grades. Ki-67 was considered positive when the nuclei of cells stained brown. The fields with the highest number of Ki-67-labeled cells were selected and a percentage of positively labeled cells were obtained. This was done by counting more than 1000 tumor nuclei using an eyepiece grid.

Statistical analysis

Analysis revealed that 16 tumors met the WHO classification for LGGs, and 22 met the classification for HGGs (**Figure 1**). LGGs were separately analyzed using Ki-67 as the grouping criterion. LGGs were segmented into LGGs with low Ki-67 LIs (<4% in diffuse astrocytoma, <5% in oligodendrogliomas, and <6% in oligoastrocytomas) and LGGs with high Ki-67 LIs (>4% in diffuse astrocytoma, >5% in oligodendrogliomas, and >6% in oligoastrocytomas) (**Figure 2**).

The difference in progression-free survival (PFS) was evaluated among tumor groups with Fisher's exact test (**Table 2**). The Spearman rank correlation coefficient was calculated to establish the relationship between the minADC values and Ki-67 LIs in LGGs and HGGs (**Figure 3**). Student's t-test was performed to determine the minADC value differences between tumor groups (LGGs with low Ki-67 LIs, LGGs with high Ki-67 LIs and HGGs) (**Table 3**; **Figure 4**). For all statistical analyses, P<0.05 was considered to
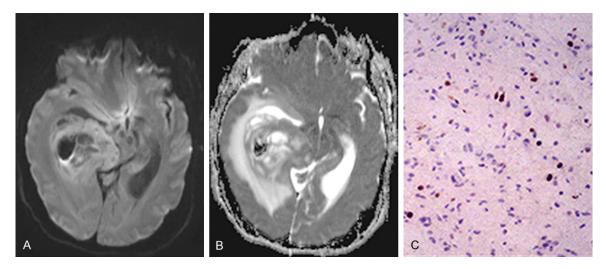
Figure 2. A 59-year-old patient with astrocytoma, WHO 2 grade. A: Low-grade glioma (LGG) with high Ki-67 was on slight hyperintense on diffusion-weighted image (b=1000 s/mm^2), B: Hypointense on apparent diffusion coefficient map (b=0, 1000 s/mm^2), C: Ki-67 staining of astrocytoma (×400), Ki-67 LI: 10%.

Table 2. The follow-up of 36 patients

Group	Follow-up	Recrudescence or dead	Progression free survival
LGG with low Ki-67 LI	9	0 (0)	9 (100%)
LGG with high Ki-67 LI	7	4 (57.1%)	3 (42.9%)
HGG	20	13 (65%)	7 (35%)

indicate significance. SPSS 17.0 (SPSS, Chicago, IL, USA) and Prism 5.0 (GraphPad, Inc) software were used in this analysis.

Results

There were 16 LGGs and 22 HGGs in our study. The LGGs had high Ki-67 LIs in 7 cases and low Ki-67 LIs in 9 cases. Due to the patient, 2 patients were withdrawn. Since initial treatment, 15 patients relapsed and/or died. A total of 1 LGG patient with a high Ki-67 LI and 1 HGG patient were dead after the surgery. A significant PFS difference was found between LGGs (75.0%) and HGGs (35.0%) (P=0.023). LGGs with low Ki-67 LIs had PFS values of 100%. LGGs with high Ki-67 LIs had PFS values of 42.9%. The difference was statistically significant (P=0.019).

We observed a strong negative association between the Ki-67 LI and minADC value for gliomas (r=-0.688, P=0.000). This finding was consistent with the hypothesis that minADC values reflect general mitotic tumor activity. The inverse association between these param-

eters was also noted when patients were split into LGGs and HGGs (LGGs, r=-0.529, P=0.035 and HGGs, r=-0.483, P=0.023). The mean minADC value for HGGs (0.69±0.25) was significantly lower than that for LGGs (0.99±0.33) (P=0.004). There was some overlap between the values for the two groups. Additionally, the mean minADC value between LGGs with low (1.17±0.32) and high Ki-67 LIs (0.76±0.18) was significantly different (P=0.008). No minADC value difference was found between LGGs with high Ki-67 LIs and HGGs (P=0.559).

Discussion

The HGGs had lower PFS and minADC values than the other groups. Many studies have reported similar findings. Higano S [23] and Calvar JA [22] also found a significant negative correlation between minADC values and Ki-67 LIs in HGG. Higano S [23] found that the minimum ADC value could predict the malignant astrocytic tumor prognosis. Other studies [24-26] have revealed that low ADC values predict worse prognoses in HGGs. However, research regarding the relationship between minADC values and prognosis has been limited.

Ki-67 LI had been used independently to assess glioma prognoses. Ki-67 is a nuclear protein expressed in the G1, S, G2, and M phas-
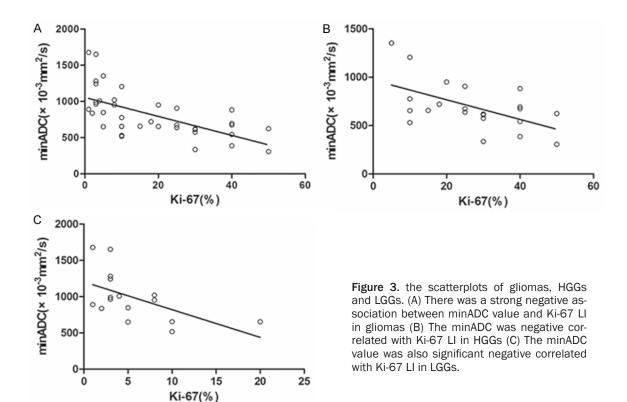
Figure 3. the scatterplots of gliomas, HGGs and LGGs. (A) There was a strong negative association between minADC value and Ki-67 LI in gliomas (B) The minADC was negative correlated with Ki-67 LI in HGGs (C) The minADC value was also significant negative correlated with Ki-67 LI in LGGs.

Table 3. Ki-67 LI and minADC value of three type gliomas

Tumor type	Number of case	Ki-67 LI (%)	MinADC value (10^{-3} mm^2/s)
LGGs with low Ki-67 LI	9 (23.7%)	2.67±1.12	1.14±0.35
LGGs with high Ki-67 LI	7 (18.4%)	18.00±27.42	0.76±0.18
HGGs	22 (57.9%)	18.00±27.42	0.69±0.25

es of the cell cycle, except for resting cells in the G0 phase. Patients had worse prognoses if they presented high proliferation activity (high Ki-67 LI). LGGs with low Ki-67 LIs had better PFS than those with high Ki-67 LIs. This result was consistent with many previous reports.

A meta-analysis [6] found a significant association between Ki-67 overexpression and worse overall survival (OS). A significant association was also found between Ki-67 overexpression and PFS in patients with glioma. Some investigators have reported the suitable prognostic value of Ki-67 for survival in LGGs. Dehghani F [9] found that patients with grade II oligodendrogliomas and Ki-67 LIs greater than 5% had longer median survival times and higher 5-year survival rates than those with grade II oligodendrogliomas and Ki-67 LIs smaller than 5%. The difference in the former was more significant

than for patients with Ki-67 LIs greater or smaller than 2%. Coon SW [16] also found that patients with Ki-67 LIs less than or equal to 5% had longer median survival than those with values greater than 5% in oligodendrogliomas. Ki67/MIB-1 proliferation indexes are also generally low, with values reported below 4 and 6% in diffuse astrocytomas and oligoastrocytomas, respectively [7, 8]. Higher Ki67/MIB-1 labeling indexes are generally associated with worse prognoses.

The prognostic value of Ki-67 was used to split LGGs into low Ki-67 LI and high Ki-67 LI groups. The differences in PFS were significant. A negative correlation between the minADC values and Ki-67 LIs was observed in LGGs, which was significant, like the HGG differences in minADC values. DW imaging can reflect the motion property of water molecules. Extracellular and intracellular environments can be influenced by pathological changes in tissue. Examples include extracellular space or cell density, tubular structures, nuclei, and nuclear tissue contour. Tumors should have rapid growth potency with high Ki-67 LIs. This potency should yield
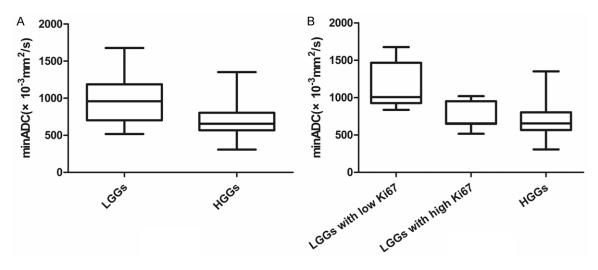
Figure 4. The box pot of each group. (A) Box pot of minADC ($\times 10^{-3}$ mm^2/s) obtained in LGGs (low-grate gliomas) and HGGs (high-grate gliomas), there was difference between them (B) there was a significant difference between LGGs with low Ki-67 LI and LGGs with high Ki-67 LI, but no difference between LGGs with high Ki-67 LI and HGGs.

high cellularity, reducing the extracellular and intracellular environments. Therefore, gliomas with high Ki-67 LIs had low minADC values.

The mean ADC value in our study did not correspond to the mean Ki-67 LI. The Ki-67 LI was obtained from one of the regions in the specimen with the highest cell density. The minADC value was taken from a region of the tumor with the highest cellularity. Higano S [23] used the same method in their retrospective study.

The Ki-67 LIs of all HGGs were greater than 5 in our study. This result partly explains the non-significant minADC value differences between LGGs with high Ki-67 LIs and HGGs. Previous studies [2, 3] have shown that certain LGGs progress more rapidly with dedifferentiation into high-grade tumors that become fatal. This difference may explain why the minADC values overlapped. We found a difference in tumor minADC values between LGGs and HGGs. Substantial overlap in the regional ADCs was found between the two, which is consistent with previous studies. Coon [16] found the median survival rate of the grade II tumors with an LI greater than or equal to 5 (620 days) to be almost identical to that of the grade III tumors. Therefore, minADC values may help distinguish LGGs with different prognoses. ADC values can also be used to predict malignant astrocytoma survival rates. DW imaging is a clinically relevant imaging biomarker with the potential to predict LGG prognoses. This technique may be incorporated into glioma prognosis evaluations.

Several limitations were present in this study. First, the study design was retrospective. No concordance between the minADC measurements in the Ki-67 LI cases was observed. Only the minADC value was included in this study. However, a potential mismatch between ADC measurements and histopathologic specimens remains. Second, the number of subjects was small. The size was inherent to our inclusion criteria. Only 7.5% of patients with LGGs had high Ki-67 LIs. Finally, the follow-up period was too short. We need to improve upon these limitations in future studies.

In conclusion, minADC tumor values were significantly correlated with Ki-67 LIs. These values differed between LGGs with high and low Ki-67 LIs. Lower ADC values indicate high glioma grades and worse prognoses in LGGs. Therefore, we believe that minADC analysis is a clinically feasible technique to predict gliomas prognosis and that it may help plan initial treatment strategies in patients with LGGs.

Acknowledgements

Disclosure of conflict of interest

None.

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