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Evaluation of histopathological changes in the microstructure at the center and periphery of glioma tumors using diffusional kurtosis imaging

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ABSTRACT

Objective: To explore the relationship between alterations in gliomas revealed by diffusional kurtosis imaging (DKI) and the histopathological microstructural changes.
Methods: Thirty-seven patients with cerebral gliomas underwent conventional MRI and DKI at 3.0 T. Normalized fractional anisotropy (FA), mean diffusivity (MD) and mean kurtosis (MK) were compared in different regions of glioma tumors. Parameters with a high sensitivity and specificity regarding the discrimination of glioma grade were evaluated using receiver operating characteristic (ROC) curve analysis. Correlations between normalized FA, MD, and MK and histopathological findings (tumor cell density, total vascular area [TVA], and Ki-67 labeling index [LI]) were assessed using Pearson correlation analyses.
Results: Normalized FA, MD, and MK differed significantly between low-grade gliomas (LGGs) and high-grade gliomas (HGGs) ($P = 0.02$, $P = 0.001$ and $P < 0.001$, respectively) at the center of the tumor. Normalized MK exhibited the highest sensitivity (80%) and specificity (100%) in distinguishing HGGs from LGGs. Relative to the tumor center, normalized MK was significantly increased in the tumor periphery ($P < 0.001$) in LGGs and significantly decreased ($P = 0.002$) in HGGs. The significant correlations were found between normalized MK and all histopathological findings (tumor cell density: $r = 0.596$, $P = 0.006$; TVA: $r = 0.764$, $P < 0.001$; and Ki-67 LI: $r = 0.766$, $P < 0.001$) among samples from the center of the tumor.
Conclusion: DKI, especially concerning the MK parameter, demonstrated high sensitivity in the detection of microstructural changes in patients with brain gliomas.

1. Introduction

Gliomas are the most common intrinsic brain tumor of the central nervous system. Glioma tissues are inhomogeneous in nature. In the process of malignant transformation, the histopathological characteristics of the tumor will change drastically, mirroring the changes in tumor microstructure [1]. Conventional MR imaging techniques have documented the macrostructural changes in gliomas [2]. However, detection of these changes at the microstructural level is limited using these techniques; for example, during the course of tumor progression gliomas exhibit increased levels of cellularity, micronecrosis and vascular hyperplasia. In addition, infiltration is often accompanied by the formation of new blood vessels, remodeling of the original vasculature, and normal brain tissue damage [3]. Information on glioma microstructure is essential for neurosurgeons to be able to choose the stereotactic biopsy site and plan the region of tumor resection. Therefore, accurate characterization and diagnosis of microstructural abnormalities in gliomas are important in a clinical setting [4]. We assessed whether there is an imaging biomarker that can be used to noninvasively depict the microstructural changes.

Diffusion imaging, including diffusion-weighted imaging (DWI), has been used for the assessment of microstructural changes in gliomas [5,6]. In addition, the widely used diffusion tensor imaging (DTI) has been shown to have high sensitivity in the detection of microstructural changes during glioma development [7–9]. However, DWI and DTI are inaccurate in monitoring cellular changes related to malignant progression, because they are both based on a Gaussian distribution of water molecules [10,11]. Because of the complexity of the microstructural environment (such as cell membranes, organelles, and water compartments), the diffusion of water molecules actually deviates from a Gaussian distribution,

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limiting the ability of conventional DWI and DTI regarding the detection of alternations in microstructure [12–14].

Diffusional kurtosis imaging (DKI) is a recently developed technique and is specifically an extension of the DTI modality. It can be used to obtain more comprehensive parameters, including all the conventional DTI diffusional parameters (fractional anisotropy [FA], axial diffusivity, radial diffusivity, and mean diffusivity [MD]), and additional diffusional kurtosis parameters (axial kurtosis (AK), radial kurtosis (RK), and mean kurtosis [MK]). MK is the average apparent kurtosis along all diffusion directions; AK and RK are defined as the kurtosis parallel and perpendicular to the principle diffusion eigenvector. MK was thought to be an indicator of microstructural complexity [12,15,16]. Non-Gaussian water diffusion behavior can be better characterized using these new parameters [17,18]. The advantage of DKI relative to DTI is that the former technique can be used not only to characterize both the gray and white matter [14,19], but also to resolve the problem of crossing neural fiber tracts [20].

Although previous studies [17,21] have shown that DKI can more effectively distinguish different grade gliomas and detect the microstructural changes (cellularity, angiogenesis, and cellular proliferation) in gliomas than conventional DWI and DTI, these studies lack pathological correlation and are limited to research on the center of tumor. For the purpose of noninvasively evaluating microstructural changes, the DKI technique was performed on 37 patients with cerebral gliomas in our study. The objective was to explore: 1) the discrimination between low-grade gliomas (LGGs) and high-grade gliomas (HGGs), and the difference between the tumor center and periphery using DKI; and 2) the correlation between DKI parameters and histopathological microstructural changes.

2. Materials and methods

2.1. Patients

The study was approved by the local institutional review board, and all of the patients voluntarily gave their signed informed consent. A total of 37 patients (14 women and 23 men; age range, 23–66 years; mean age, 47 years) with pathologically confirmed gliomas, who were treated in our hospital or were diagnosed in our outpatient clinic between June2014 and August 2015, were enrolled in the study. The patients underwent surgery within 7 days after the MR examination. All patients had not undergone surgery, chemotherapy, or radiotherapy. All tissue specimens were examined by an experienced neuropathologist. The grade of gliomas was determined according to the World Health Organization (WHO) classification. There were 16 LGGs of WHO grades I and II (one pilocytic astrocytoma, six oligodendrogliomas, and nine diffuse astrocytomas), and 21 HGGs of WHO grades III and IV (four anaplastic astrocytomas, two anaplastic oligodendroglioma, and 15 glioblastomas).

2.2. MR imaging methods

MR examinations were performed on all patients using a 3.0 T MR imager (MagnetomVerio: Siemens, Germany). The DKI experiments were performed using diffusion-weighted pulse sequence. DKI data were acquired using 6 *b* values (0, 500, 1000, 1500, 2000, and 2500 s/mm^2) and 30 different diffusion encoding directions [12,17]. Other imaging parameters were: repetition time ms/echo time ms, 3000/109; field of view, 256 mm × 256 mm; matrix, 128 × 128; number of signals acquired, two; number of sections, 15; section thickness, 4 mm; imaging time, 15 minutes17 s.

All patients also underwent routine MR imaging, which was used in post-processing and served as an anatomical reference for DKI. The routine MR imaging scans consisted of transverse T2 turbo spin-echo (T2- TSE)(4000/93; field of view, 220 mm × 184 mm; matrix, 256 × 256; intersection gap, 1 mm; 20 sections; section thickness, 6 mm) and the contrast-enhanced 3D T1-weighted fast low-angle shot (FLASH) (19/4.92; field of view, 250 mm × 170 mm; matrix, 256 × 256; flip angle, 25; imaging time, 3 min 48 s) performed before and after the injection of gadopentetate dimeglumine (Magnevist; Schering, Berlin, Germany)at a dose of 0.1 mm/kg body weight.

2.3. Image processing and data analysis

DKI post-processing was performed using the Diffusional Kurtosis Estimator (http://www.nitrc.org/projects/dke) [22]. The parametric maps of FA, MD, and MK were obtained using the software. Before drawing the regions of interest (ROIs), the contrast-enhanced 3D T1-FLASH and T2-FSE images were coregistered and resliced to the DKI metric maps by running modules developed in-house in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) and Matlabr2009a (MathWorks, Natick, MA, USA) [23]. The ROIs were manually drawn around the tumor center, tumor periphery, and contralateral normal-appearing white matter (NAWMc) by an experienced neuroradiologist (blinded to the pathological results) using MRIcro (http://www.nitrc.org/projects/mricron). The ROIs over the solid enhancing tumor were outlined according to the contrast-enhanced 3D T1-FLASH images, avoiding cystic components, hemorrhages, necrosis, and calcifications; the ROIs over the non-enhancing tumor were delineated according to the transverse T2-FSE, and the ROIs over NAWMc were delineated according to the FA maps. In the HGGs, the tumor center was defined as the area of enhancement; ROIs for the peritumoral region were placed into 2-mm bands around the outer tumor center [24]. In the LGGs that were not enhanced, the tumor center was defined as the area of increased T2-signal; for the peritumoral region, ROIs were positioned in the 2-mm bands around the outer region with increased T2-signal. For the NAWMc, ROIs were positioned in the white matter of the contralateral hemisphere to coincide with the location of the lesion, avoiding the gray matter (Fig. 1). The ROIs were copied from the contrast-enhanced 3D T1-FLASH or T2-FSE images to all DKI metric maps. We calculated FA, MD, and MK values for every ROI using the MRIcro software. The parameter values for the tumor center and tumor periphery were normalized to the corresponding values in the NAWMc of each patient as follows: normalized MK = MK (tumor center or tumor periphery)/MK (NAWMc). The other two normalized metrics (normalized FA and MD) were also calculated using the same method as that used for normalized MK to eliminate whole-brain inter-individual variations [17,21,25].

2.4. Pathology and immunohistochemistry

To ensure accurate correspondence between the location of the histopathological samples and the MR images, we requested that the neurosurgeon obtain tissue from the tumor center and peritumoural region determined preoperatively via conventional MR images. The samples from each patient were fixed in 4% paraformaldehyde for 48 h, embedded in paraffin wax, sectioned at 4 um, and stained with hematoxylin-eosin.

Immunohistochemical staining with Ki-67, CD34, and microtubule-associated protein 2 (MAP2) antibodies was performed on all samples using the Strept Avidin-Biotin Complex method and diaminobenzidine as chromogen. In the negative controls, the primary antibodies were replaced with phosphate buffer saline. The primary antibodies were: Ki-67 (Abcam, monoclonal,