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ORIGINAL ARTICLE

Significance of nuclear magnetic resonance combined with Ki-67 and VEGF detection in the diagnosis and prognosis evaluation of brain glioma

Xuexia Yuan[1]*, Deguo Liu[1]*, Yanhui Wang[1], Xiaoxia Li[2]

[1]Medical Image Center and [2]Department of Pathology, Affiliated Hospital of Jining Medical University, Jining, Shandong 272029, China

*These authors contributed equally to this work.

Summary

Purpose: *To investigate the significance of nuclear magnetic resonance (NMR) combined with Ki-67 and vascular endothelial growth factor (VEGF) detection in the diagnosis and prognosis evaluation of brain glioma.*

Methods: *78 patients with brain glioma treated at the Affiliated Hospital of Jining Medical University from January 2015 to August 2017 were studied. All patients underwent the NMR diffusion tensor imaging examination. The expressions of Ki-67 and VEGF in brain glioma tissues were detected using immunohistochemistry and survival analyses were performed for patients in high apparent diffusion coefficient (ADC) group and low ADC group, high-expression Ki-67 and VEGF group and low-expression Ki-67 and VEGF group before treatment. Moreover, the value of combined diagnosis in the prognosis evaluation of patients was analyzed.*

Results: *NMR diffusion tensor imaging showed that the fractional anisotropy (FA) value in the tumor enhancement region on the affected side was significantly lower than in the contralateral normal region, but ADC was significantly higher in the contralateral normal region; the FA value in grade I-II brain glioma enhancement region was higher compared with grade III-IV glioma enhancement region, but ADC was lower in the grade-III-IV glioma enhancement region ($p<0.05$). The low expression rates of Ki-67 and VEGF in patients with grade I-II brain glioma were significantly higher than in patients with grade III-IV glioma ($p<0.05$). After treatment, the 3-year survival rate of high ADC group was lower than that of low ADC group, and the 3-year survival rate of high-expression Ki-67 and VEGF group was also obviously lower than that in low-expression Ki-67 and VEGF group ($p<0.05$). Besides, the area under the receiver operating characteristic (ROC) curve of NMR combined with Ki-67 and VEGF detection in the prediction of patient prognosis was 0.906, the sensitivity 91.6%, and the specificity 89.5%.*

Conclusion: *NMR diffusion tensor imaging has a high application value in the diagnosis of brain glioma. The expressions of Ki-67 and VEGF are related to the pathological grade of glioma, which can be used as biological indexes for the diagnosis of glioma. Moreover, the combined detection of the three items can not only accurately determine the grade of glioma malignancy, but also effectively evaluate the prognosis of patients, thus providing a scientific basis for the selection of therapeutic regimen.*

Key words: *brain glioma, Ki-67, nuclear magnetic resonance diffusion tensor imaging, VEGF*

Introduction

Brain glioma is a common tumor of the central nervous system, accounting for about 50% of intracranial tumors [1]. The treatment methods of brain glioma include radiotherapy, chemotherapy, surgical treatment, gene therapy and molecular biological therapy. Surgical treatment is dominant,

Correspondence to: Dr. Deguo Liu, Guhuai Road no. 89, Jining City, Shandong Province 272029, China.
Tel and Fax: +86 01515377300, E-mail: 123opqw@sina.com
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however, the disease cannot be completely resected. Besides, glioma will relapse in many instances after operation and the patient survival is short [2]. Brain glioma mostly shows infiltrative growth and diffuse distribution, its clinical manifestations lack specificity, and the diagnosis is difficult without biopsy, so it is particularly important to choose the appropriate diagnostic methods and reasonable treatment [3]. NMR diffusion tensor imaging is a diagnostic technique emerged in recent years, which is widely used in the diagnosis of central nervous system diseases. Moreover, it can accurately evaluate the microstructure of local areas through diffusion quantification of water molecules in the brain [4]. Ki-67 is clinically used as a marker for the detection of tumor cell proliferation [5]. VEGF is a growth factor of vascular endothelial cells, which can promote the tumor growth, invasion and metastasis [6]. In this study, patients with brain glioma were subjected to NMR combined with Ki-67 and VEGF detection, and their prognosis was analyzed, providing a scientific basis for the diagnosis and treatment of the disease.

Methods

General data

A total of 78 patients with brain glioma treated in the Affiliated Hospital of Jining Medical University from January 2015 to August 2017 were randomly selected. Inclusion criteria: 1) patients meeting the diagnostic criteria of brain glioma and confirmed via pathological diagnosis [7]; 2) patients with predicted survival >8 weeks and unilateral lesions; 3) patients who signed informed consent. Patients with contraindications of MRI examination were excluded, and the general data of patients are shown in Table 1.

Table 1. General patient & disease characteristics

Characteristics	*Patients (n=78) mean±SD*
Age, years	45.78±7.54
Gender (male/female)	43/35
Kamofsky performance status score	70.21±4.78
Average tumor diameter (mm)	31.63±1.52
Pathological grade n, (%)	
Grade I-II	41 (52.56)
Grade III-IV	37 (47.44)

Preparations before examination

Patients had a light diet one day before NMR examination to avoid signal changes due to high-fat foods, while they received psychological counseling to ensure a calm mood.

NMR diffusion tensor imaging examination

NMR diffusion tensor imaging examination was performed using the Sigma3.0T NMR scanner (GE). Patients were put at supine position and asked to keep quiet breathing; the head was immobilized as far as possible and the swallowing was avoided. Besides, they underwent T1W1 (TR: 360 ms; TE: 20 ms; matrix: 385×190; FOV: 23 cm×17 cm; layer spacing: 3 mm; layer thickness: 7 mm), T2W1 (TR: 220 ms; TE: 21 ms; matrix: 482×230; FOV: 23 cm×18 cm; layer spacing: 3 mm; layer thickness: 7 mm) and fluid-attenuated inversion-recovery (FLAIR) scanning (TR: 5000 ms; TE: 20 ms; matrix: 385×190; FOV: 23 cm×17 cm; layer spacing: 3 mm; layer thickness: 7 mm), followed by image optimization using the system-provided image processing tools.

Ki-67 and VEGF detection

The expressions of Ki-67 and VEGF in brain glioma specimens were detected using immunohistochemistry. The rabbit anti-Ki-67 polyclonal antibody and VEGF rabbit anti-human polyclonal antibody were supplied by Beijing Bioss Biotechnology Co., Ltd. The paraffin-embedded tissues were cut into 4 μm-thick sections using slicer (Leica, Germany); then the sections were placed into 95, 90, 85 and 75% ethanol for 10 min, respectively, soaked in distilled water for 5 min, and washed with phosphate buffered saline (PBS) for 3 times (5 min/time); 50 μL primary antibody was added and the sections were placed at 4°C overnight, and then the secondary antibody was added for incubation at 20°C for 15 min. The reagents A, B and C in diaminobenzidine (DAB) kit (Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd.) were added in the sections for color development, followed by observation under microscope. After color development, the reagents were washed off with distilled water to terminate the development, followed by the hematoxylin re-staining for 2 min and sealing via neutral gum.

Evaluation indexes

The changes of ADC and FA values in the tumor region and the contralateral normal white matter of patients were observed, and the ADC and FA value in the tumor enhancement region of patients with different pathological grades were compared.

The expressions of Ki-67 and VEGF in tumor tissues were detected using the immunohistochemistry and the cells stained brown-yellow indicated positivity. The percentage of positive cells was calculated in 4 high-power fields (×400) randomly selected from each section, and the percentage point (PP) was scored: 1) 0 point: no positive cells; 2) 1 point: positive cells <5%; 3) 2 points: positive cells <5% - ≤20%; 4) 3 points: positive cells >20%. The staining intensity (SI) was also scored: 1) 0 points: no staining; 2) 1 point: faint yellow; 3) 2 points: brown-yellow; 4) 3 points: dark-brown. The formula used was as follows: immunoreaction score (IRS)= PP×SI. IRS >4 was defined as high expression, while IRS ≤4 as low expression [8].

Statistics

The Statistical Package for Social Sciences (SPSS) 19.0 (SPSS Inc., Chicago, IL, USA) was used. Quantitative data were presented as mean±standard deviation and analysed with the t-test. Numerical data were presented as percents and analysed with the chi-square test. ROC curve analysis was performed for the NMR combined with Ki-67 and VEGF detection for the evaluation of prognosis, and survival analysis was performed via Kaplan-Meier analysis and log-rank test. A p value <0.05 was considered as statistically significant.

Results

The NMR diffusion tensor imaging examination of each region of brain glioma showed that the FA value in the tumor enhancement region on the affected side was lower than that in the contralateral normal region, but ADC was significantly higher than that in the contralateral normal region (p<0.05; Table 2).

According to the comparisons of brain glioma in different pathological grades, the FA value in the grade I-II brain glioma enhancement region was obviously higher than in the grade III-IV glioma enhancement region, but ADC was obviously lower than in the grade III-IV glioma enhancement region (p<0.05; Table 3).

Comparisons of Ki-67 expressions in brain glioma in different pathological grades showed that high expression rate of Ki-67 in patients with grade I-II brain glioma was significantly lower than in patients with grade III-IV brain glioma (p<0.05; Table 4).

Comparisons of VEGF expressions in brain glioma in different pathological grades showed that high expression rate of VEGF in patients with grade-I-II brain glioma was significantly lower compared with patients with grade III-IV brain glioma (p<0.05; Table 5).

After treatment, the 3-year survival rate of high ADC (>0.6) group was significantly lower compared with low ADC group (≤0.6) (p<0.05), and the 3-year survival rate of high-expression Ki-67 and VEGF group was also significantly lower than that in the low-expression Ki-67 and VEGF group (p<0.05; Figures 1, 2 and 3). The area under the ROC curve of NMR combined with Ki-67 and VEGF detection in the prediction of prognosis of patients was 0.906, the sensitivity 91.6%, and the specificity 89.5% (Figure 4).

Table 2. Comparisons of brain glioma detection results

	FA mean±SD	*ADC mean±SD*
Tumor enhancement region on the affected side	0.18±0.03	0.59±0.07
Contralateral normal region	0.57±0.06	0.31±0.05
t	51.346	28.747
p	<0.001	<0.001

FA: fractional anisotropy, ADC: apparent diffusion coefficient

Table 3. Comparisons of detection results of brain glioma in different pathological grades

Pathological grade	*FA mean±SD*	*ADC mean±SD*
I-II	0.25±0.04	0.57±0.05
III-IV	0.16±0.02	0.69±0.06
t	17.774	13.569
p	<0.001	<0.001

For abbreviations see footnote of Table 2

Table 4. Ki-67 expressions in brain glioma in different pathological grades

Pathological grade	*Low expression n (%)*	*High expression n (%)*
I-II	57 (73.08)	21 (26.92)
III-IV	17 (21.79)	61 (78.21)
x^2		39.103
p		<0.001

Table 5. VEGF expressions in brain glioma in different pathological grades

Pathological grade	*Low expression n (%)*	*High expression n (%)*
I-II	56 (71.79)	22 (28.21)
III-IV	26 (33.33)	52 (66.67)
x^2		21.621
p		<0.001

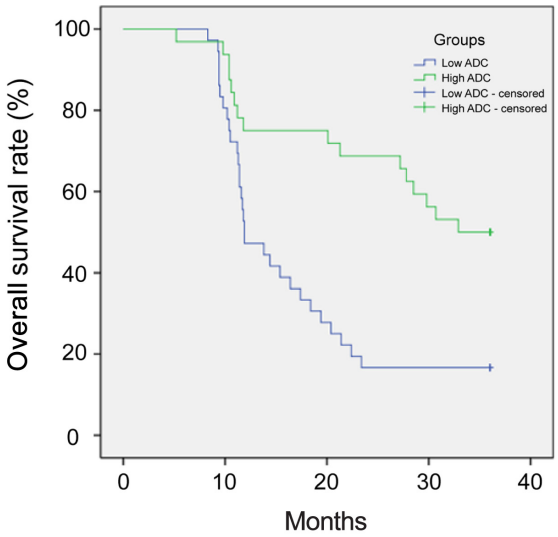

Figure 1. Kaplan-Meier overall survival of patients with different ADCs (p<0.05).

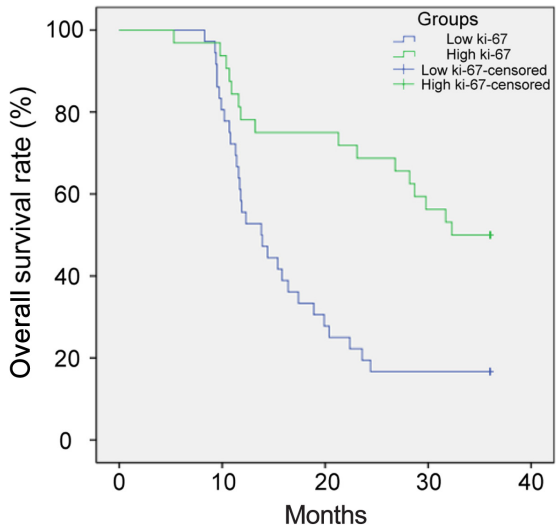

Figure 2. Kaplan-Meier overall survival of patients with different Ki-67 expressions (p<0.05).

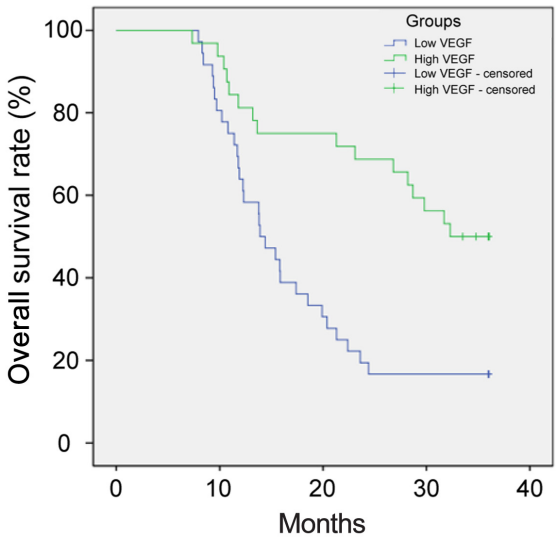

Figure 3. Kaplan-Meier overall survival of patients with different VEGF expressions (p<0.05).

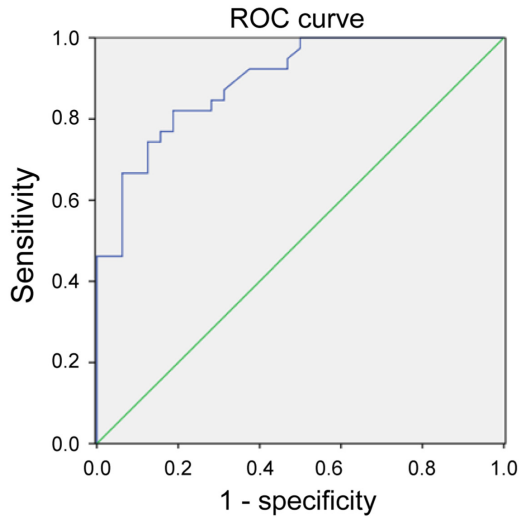

Figure 4. The area under the ROC curve of NMR combined with Ki-67 and VEGF detection in the prediction of patient prognosis was 0.906, the sensitivity was 91.6% and the specificity 89.5%.

Discussion

Gliomas are divided into oligodendroglioma, astrocytoma, ependymoma, oligoastrocytomas, glioblastoma, etc [1]. In order to facilitate the research, it is usually divided into high-grade brain glioma (grades III-IV) and low-grade glioma (grades I-II) [9]. The clinical manifestations of brain glioma include headache, malignant vomiting, neurological deficits, optic fundus edema, hemiplegia, changes in mental status and ataxia, and it is difficult to be cured; besides, patients have poor quality of life and a high mortality rate [10]. Brain glioma is mainly diagnosed by clinical characteristics, imaging analysis and biopsy [11]. Surgery is the preferred treatment of glioma, but due to the highly malignant behavior of this disease and the postoperative recurrence, the results of treatment are largely unsatisfactory, seriously affecting the prognosis and patient life, so it is of great significance to see for more effective detection means in its diagnosis, treatment and evaluation of prognosis [12].

Diffusion tensor imaging is a method in NMR of describing the brain structure, which can be used to assess the brain glioma through various parameters [13]. The diagnostic principle of NMR diffusion tensor imaging is that the free water molecules in the brain can be diffused and the diffusion degree is affected by the structure of the brain. In particular, the white matter will be different due to the influence of the lesion [14]. FA and ADC are the main parameters of diffusion tensor imaging, in which FA is the proportion of FA of free water molecules in the total diffusion tensor and can reflect the diffusion limitation, and ADC can reflect the diffusion activity of water molecules [15]. The results of this study showed that the FA value in the tumor enhancement region on the affected side was significantly lower than in the contralateral normal region, but ADC was higher in the contralateral normal region; the FA value in grade I-II brain glioma enhancement region was significantly higher than in the grade III-IV glioma enhancement region, but ADC was significantly lower in the grade III-IV glioma enhancement region (p<0.05), suggesting that the diffusion limitation on the affected side and high-grade glioma enhancement region is weaker, the tumor cell density is higher and the infiltration is more serious, because the tumor region has serious edema, large cell density and small extracellular space, thus affecting the diffusion of free water molecules and ADC [16]. In addition, this study showed that after treatment, the 3-year survival rate of the high ADC group was significantly lower compared with the

low ADC group (p<0.05), because the higher ADC led to higher tumor cell density, more rapid cell proliferation, and more serious damage to the surrounding normal brain tissues, thus resulting in rapid disease progression and poor prognosis.

VEGF is an angiogenic factor with the highest specificity and strongest active function, as well as a member in the platelet-derived growth factor family, which can stimulate the vascular endothelial cells, thereby promoting their division and proliferation, and ultimately promoting the neovascularization [17]. VEGF is highly expressed in various tumor tissues and is closely related to the pathological grade of malignant tumors [18]. The results of this study revealed that the high expression rate of VEGF in patients with grade I-II brain glioma was significantly lower than in patients with grade III-IV disease, and the 3-year survival rate of high-expression VEGF group was also significantly lower than in low-expression VEGF group (p<0.05). This is because VEGF can stimulate the proliferation of endothelial cells, increase the vascular permeability, and provide nutritional support for the establishment of new blood capillary network, thereby inducing the formation of new blood vessels, resulting in tumor growth, invasion and metastasis, and leading to continuous deterioration of glioma, which has a close relationship with its pathological grade. In addition, VEGF can also interfere and inhibit the dendritic cells, thus blocking the B and T cell antigen presentation, and affecting the normal immune mechanism, which offers immune escape in tumor cells and results in very poor prognosis of patients [19].

Ki-67 is a proliferating cell nuclear antigen found and named in the 1980s, which is related to the cell cycle. Ki-67 has been used up until now as an important biomarker to explore the proliferation of malignant tumor cells [20]. The results of this study showed that the high expression rate of Ki-67 in patients with grade I-II brain glioma was significantly lower compared with patients with grade III-IV glioma, and the 3-year survival rate of high-expression Ki-67 group was also significantly lower than that in the low-expression VEGF group (p<0.05). So, NMR combined with Ki-67 and VEGF detection can effectively diagnose the brain glioma with a high sensitivity to prognosis evaluation.

Conclusions

In conclusion, NMR combined with Ki-67 and VEGF detection for patients with brain glioma can not only diagnose the disease, but also intuitively judge the pathological grade and proliferative activity of glioma, so as to provide a basis for the rational formulation of a therapeutic regimen and prognosis evaluation.

Conflict of interests

The authors declare no conflict of interests.

References

1. Li SJ, Zhou J, Zhang L et al. The effect of miR-21 on SWOZ2 glioma cells and its biological mechanism. JBUON 2017;22:468-73.
2. Eckel-Passow JE, Lachance DH, Molinaro AM et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. N Engl J Med 2015;372:2499-2508.
3. Zhu R, Chen X, Li J, Lan L. Human glioma cells-conditioned medium promotes proliferation and migration of human bone marrow mesenchymal stromal cells in vitro. JBUON 2017;22:730-4.
4. Lee Y, Kim H. Assessment of diffusion tensor MR imaging (DTI) in liver fibrosis with minimal confounding effect of hepatic steatosis. Magn Reson Med 2015;73:1602-8.
5. Hong MK, Laskin WB, Herman BE et al. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. Cancer 1995;75:423-9.
6. Zhao D, Pan C, Sun J et al. VEGF drives cancer-initiating stem cells through VEGFR-2/Stat3 signaling to upregulate Myc and Sox2. Oncogene 2015;34:3107-19.
7. Li XT, Tang W, Jiang Y et al. Multifunctional targeting vinorelbine plus tetrandrine liposomes for treating brain glioma along with eliminating glioma stem cells. Oncotarget 2016;7:24604-22.
8. Jagadish N, Parashar D, Gupta N et al. A novel cancer testis antigen target A-kinase anchor protein (AKAP4) for the early diagnosis and immunotherapy of colon cancer. Oncoimmunology 2016;5:e1078965.
9. Karunamuni R, Bartsch H, White NS et al. Dose-Dependent cortical thinning after partial brain irradiation in High-Grade glioma. Int J Radiat Oncol Biol Phys 2016;94:297-304.
10. Stummer W. Fluorescein in brain metastasis and glioma surgery. Acta Neurochir (Wien) 2015;157:2199-2200.

11. Tonoyan AS, Pronin IN, Pitshelauri DI et al. A correlation between diffusion kurtosis imaging and the proliferative activity of brain glioma. Zh Vopr Neirokhir ImNN Burdenko 2015;79:5-14.
12. Zhang B, Zhang Y, Liao Z et al. UPA-sensitive ACPP-conjugated nanoparticles for multi-targeting therapy of brain glioma. Biomaterials 2015;36:98-109.
13. Correa DG, Zimmermann N, Doring TM et al. Diffusion tensor MR imaging of white matter integrity in HIV-positive patients with planning deficit. Neuroradiology 2015;57:475-82.
14. Lee HY, Na DG, Song IC et al. Diffusion-tensor imaging for glioma grading at 3-T magnetic resonance imaging: Analysis of fractional anisotropy and mean diffusivity. J Comput Assist Tomogr 2008;32:298-303.
15. Li X, Liang Q, Zhuang L et al. Preliminary study of MR diffusion tensor imaging of the liver for the diagnosis of hepatocellular carcinoma. PLoS One 2015;10:e135568.
16. Tonoyan A, Pronin IN, Pitskhelauri DI et al. Diffusion kurtosis imaging in diagnostics of brain glioma malignancy. Am J Orthodont 2015;77:227-9.
17. Ciamporcero E, Miles KM, Adelaiye R et al. Combination strategy targeting VEGF and HGF/c-met in human renal cell carcinoma models. Mol Cancer Ther 2015;14:101-10.
18. Hamnvik OP, Choueiri TK, Turchin A et al. Clinical risk factors for the development of hypertension in patients treated with inhibitors of the VEGF signaling pathway. Cancer 2015;121:311-9.
19. Sahara M, Hansson EM, Wernet O, Lui KO, Spater D, Chien KR. Manipulation of a VEGF-Notch signaling circuit drives formation of functional vascular endothelial progenitors from human pluripotent stem cells. Cell Res 2015;25:148.
20. Erdogan B, Turkmen E, Yalta TD et al. Importance of Ki-67 in human epidermal growth factor receptor 2 positive breast cancer. JBUON 2015;20:730-6.