ORIGINAL ARTICLE

Analysis of Relative Apparent Diffusion Coefficient Value of Intracranial Primary Central Nervous System Lymphoma and its Correlation with Ki-67 Positive Rate

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ABSTRACT

Objective: The objective of the study was to study the change of relative apparent diffusion coefficient (rADC) value in intracranial primary central nervous system lymphoma (PCNSL) in magnetic resonance diffusion-weighted imaging (DWI) and analyze its correlation with Ki-67 positive rate. **Materials and Methods:** Fifty cases of intracranial PCNSL patients treated in our hospital from May 2015 to May 2017 were selected as the PCNSL group, and 50 cases of glioblastoma (GBM) patients were the GBM group. Magnetic resonance DWI was used to analyze the rADC value of each group. Meanwhile, immunohistochemical method was used to detect Ki-67 expression in each group, and the correlation between the rADC value and Ki-67 in each group was also investigated. **Results:** The rADC values in patients with PCNSL (0.69 ± 0.08) $\times 10^{-5}$ m^2/s were significantly lower than those in patients with GBM (0.78 ± 0.03) $\times 10^{-5}$ m^2/s. There was a significant difference between the two groups ($P < 0.05$). The positive rates of Ki-67 in the PCNSL group and GBM group were 92.0% and 62.0%, respectively, with significant difference between the two groups ($P < 0.05$). According to Spearman rank correlation analysis, there was a negative correlation between the rADC values in the PCNSL group and GBM group (r PCNSL group = −0.607, $P < 0.05$); r GBM group = −0.613, $P < 0.05$). **Conclusion:** The diagnosis of intracranial PCNSL and GBM with DWI has differential value. The value of rADC is negatively correlated with Ki-67 positive rate, which provides a non-invasive quantitative index for the judgment of the deterioration degree of PCNSL and GBM.

Key words: Glioblastoma, Ki-67, primary central nervous system lymphoma, relative apparent diffusion coefficient

INTRODUCTION

Primary central nervous system lymphoma (PCNSL) is a non-Hodgkin lymphoma that occurs only in the brain and spinal cord without systemic lymphadenopathy or infiltration of lymphoid tissue. PCNSL is almost all non-Hodgkin lymphoma, and most of them are B lymphocyte type. A few are T-lymphocyte type or non-T, non-B-cell type. Pathological type is mainly diffuse large or non-cleaved cells. Diffuse large B-cell lymphoma is most common.[1-2] Although PCNSL accounts for only about 1–5% of all primary intracranial tumors, its strong invasiveness often leads to poor prognosis. Even those with immunocompetent patients have a median survival of only about 9 months after receiving radiotherapy.[3] Consequently, the efforts to improve the diagnostic accuracy and the prognosis of PCNSL patients has been one of the hot spots of scholars at home and abroad.[4] Glioblastoma (GBM) is a highly malignant tumor of astrocyte differentiation. The incidence of GBM is about 10%. The 2 tumors have similar characteristics such as rich

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cellular density, abundant blood supply, and invasiveness. Moreover, the routine magnetic resonance image (MRI) examination is difficult to identify the atypical PCNSL and GBM.[5] However, the clinical treatment strategies of PCNSL and GBM are very different. The identification of two kinds of tumors before operation has important guiding significance for clinical treatment. The value of PCNSL and GBM is limited by routine MRI sequence examination.[6] Diffusion-weighted image (DWI) can quantitatively reflect the microscopic changes of tumor, reflect the biological characteristics of tumors, and improve the diagnosis and treatment of tumors. However, the relative apparent diffusion coefficient (rADC) obtained by computer simulation from DWI scan is relatively few in the diagnosis value of PCNCL and GBM. The expression of Ki-67 is closely related to the growth, proliferation, and metastasis of tumor cells.[7] In view of this, this study compares the difference of rADC and Ki-67 positive rates between intracranial PCNSL and GBM in DWI detection. At the same time, we will further explore the intrinsic relationship between the rADC value and Ki-67 positive rate, rADC value, and pathological characteristics, so as to provide non-invasive quantitative indicators for the judgment of the severity of the disease and improve the accuracy of clinical-pathological diagnosis.

MATERIALS AND METHODS

Clinical features of patients

Fifty cases of intracranial PCNSL patients treated in the Division of Neuro-oncology at Sungkyunkwan University, Samsung Changwon Hospital from May 2015 to May 2019 were selected as the PCNSL group, and 50 cases of GBM patients were the GBM group. Inclusion criteria: (1) The patients were diagnosed as PCNSL and GBM by neuropathology; (2) the patient did not undergo any treatment before the DWI examination; (3) ADC images of patients with tumor solid part of the area are greater than 1.0 cm^2; (4) the patients were the first symptoms, and the lesion region is confined to the central nervous system (pia mater, eye, spinal cord, and brain tissue); (5) after bone marrow puncture, routine blood tests, abdominal ultrasound, computed tomography, chest X-ray, and liver function tests, no other organ involvement and abnormal hematopoietic function were found; and (6) the patients and their families were informed of the study and voluntarily signed the informed consent. In PCNSL group, there were 30 male patients and 20 female patients in 50 cases, and the age was from 34 to 72 years old, with a mean age at 56.3 ± 2.2 years old. In the GBM group, there were 29 male patients and 21 female patients in 50 cases, and the age was from 35 to 73 years old, with a mean age at 56.9 ± 2.6 years old. There was no significant difference in gender and age between the two groups ($P > 0.05$).

Magnetic resonance DWI examination

The subjects were supine in the center of the coil. The tamponade was used to fill the whole body around the extremities, and the head was fixed. The patient was reminded to keep the limbs immobilized as far as possible and informed the patient that the examination time could be longer. The patients were examined after consent and cooperation. DWI scan: The patient was subjected to a regular MRI scan using a whole-body magnetic resonance scanner (Philips Achieva 1.5T), mainly including: T2WI transaction, TlWI, coronal and sagittal position, and the relative position of the lipid scanning. The parameters are set as follows: T2WI: Time of echo (TE) 90–100 ms, time of repetition (TR) 2500–3000 ms; TlWI: TE 8–10 ms, TR 1500–2000 ms. Single-shot echo-planar imaging sequences and fat suppression were used. The parameters are set as follows: TE 90 ms, TR 5100 ms, layer spacing of 1.0 mm, layer thickness of 5.0 mm, and the matrix is 128 × 128, FOV and mature according to the lesion to adjust, with the acquisition time of 32 s/min. The mass sensitive gradient values are 0 s/mm^2, 300 s/mm^2, and 600 s/mm^2, respectively. The mass gradient is applied to the phase encoding direction (P), the frequency encoding direction (R), and the slice selection direction (s), and the final instrument automatically generates the ADC image. Image post-processing: Region of interest (ROI) extraction selection: Three ROIs were selected to avoid the surrounding edema area, necrosis, and intratumoral vascular area as far as possible. The ROI in the parenchyma of each lesion was measured and the minimum value of ADC in the lesion area was recorded. In addition, the same size of ROI was selected in the contralateral area to calculate the ADC average of the contralateral white matter area. The rADC value is the ratio of the lowest ADC value in the lesion area to the average ADC value in the contralateral white matter area.

Pathological examination

The collected cancer tissues and precancerous tissues were prepared into paraffin sections by formalin fixation and paraffin embedding. First, the slices are dewaxing and hydrated. The uranyl uranium solution (pH = 6) was used to repair it for 2 min; then, 30 mL of hydrogen peroxide was adopted to block it for 10 min at room temperature so as to destroy the peroxides activity. After washing it with a phosphate buffer solution (PBS) for 3 times, the fetal bovine serum (50 mL) was added to seal it for 20 min, then followed by rabbit anti-human KLF4 polyclonal antibody and goat anti-rabbit lgG were incubated for 2 h and 30 min at 37° a conditions, each incubation after washing with PBS buffer solution 3 times. Finally, the horseradish peroxidase-labeled biotin was used for staining, and diaminobenzidine solution was used for coloration. After hematoxylin was stained, dehydration, transparence, and sealing were observed.

The staining of each tissue was observed with a microscope. A semi-quantitative analysis of Ki-67 was performed based on the staining intensity of the final cell and the coloration ratio. According to the rate of positive cells and the degree of colorimetric evaluation: The number of positive cells was

<14% as negative, expressed as (−); the number of positive cells from 4% to 50% was weak positive, expressed as (+), and the number of positive cells from 51% to 75% was strong positive, which is indicated by (+ +).

Statistical analysis

The SPSS 21 statistics software (IBM Corp., Armonk, NY) was used to analyze the data, and the measurement data were expressed with mean value ± standard deviation, and *t*-test was used for the comparison between the groups. The count data were expressed as percentage (%), and the comparison between them was conducted using Chi-square test. The non-parametric method was used to fit the receiver operating characteristic curve to determine the ADC cutoff value. The relationship between the rADC value and Ki-67 expression was detected using Spearman rank correlation test, $P < 0.05$ was considered statistically significant.

Ethical statement

The Institutional Review Board (IRB) of Sungkyunkwan University, Samsung Changwon Hospital approved the study protocol (IRB number: SCMC 2018-12-006). All studies were conducted according to the guidelines of the Declaration of Helsinki for biomedical research. Informed consent was waived due to its retrospective nature.

RESULTS

Comparison of magnetic resonance DWI features between two groups

In the patients of the PCNSL group, 45 patients showed high signal in the DWI image of the lesion, and the DWI image of the lesion in five patients showed the medium and high signal. Moreover, the mean value of ADC was significantly lower than that in the contralateral area [Figure 1b], and the lesion area was obviously enhanced after the enhancement [Figure 1a].

In the GBM group, 46 patients showed high signal in DWI image, 4 cases showed slightly higher signal in DWI image, and the mean value of ADC was significantly lower than that in the contralateral area [as depicted in Figure 2b], after enhancement, the substantial part of the lesion area was obviously strengthened and changed [Figure 2a].

The rADC value of the PCNSL group was $(0.69 \pm 0.08) \times 10^{-5}$ m^2/s, which was lower than that of the GBM group $(0.78 \pm 0.03) \times 10^{-5}$ m^2/s, and the difference between the two groups was statistically significant ($P<0.05$), as shown in Table 1.

Comparison of the difference of Ki-67 expression in patients of two groups

Comparing the rate of Ki-7 positive cells in different tissues, it was found that the rate of positive cells in the PCNSL group was 92%, while that in the GBM group was 62%, the difference between the two groups was statistically significant ($P < 0.05$) [Table 2]. The positive expression of Ki-67 with immune histochemical staining is illustrated in Figure 3 (PCNSL) and Figure 4 (GBM).

Correlation between rADC values and Ki-67 expression in patients of two groups

There was a negative correlation between the rADC values and Ki-67 positive rate in the PCNSL group and GBM group by Spearman's rank correlation analysis between the rADC value and Ki-67 positive rate (r PCNSL group = −0.607, $P < 0.05$; r GBM group = −0.613, $P < 0.05$).

In brief summaries of the results, the rADC values in patients with PCNSL $(0.69 \pm 0.08) \times 10^{-5}$ m^2/s were significantly lower than those in patients with GBM $(0.78 \pm 0.03) \times 10^{-5}$ m^2/s. There was a significant difference between the two

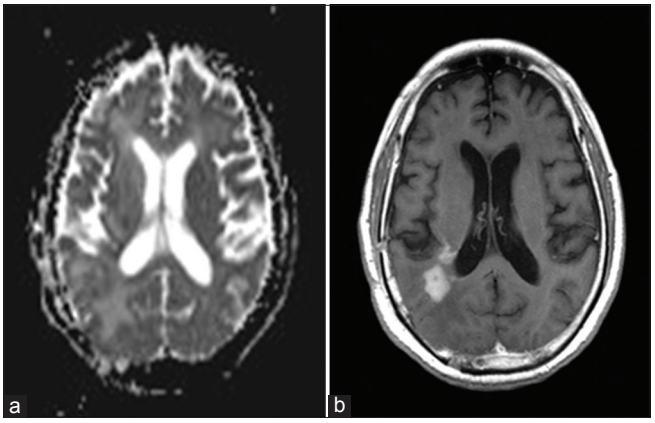

Figure 1: Magnetic resonance images of PCNSL patients. (a) The axial image of ADC showed that the parenchyma of the right parietal lesion was markedly enhanced. (b) At the corresponding level of ADC image, the enhanced part of the lesion showed low signal in the ADC image

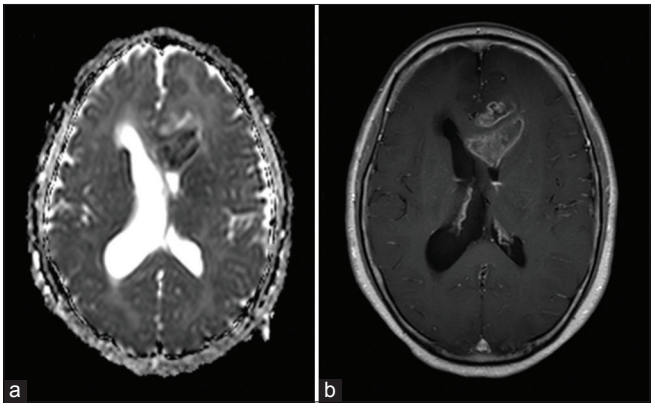

Figure 2: Magnetic resonance images of GBM patients. (a) The axial position image of ADC showed that the parenchyma of the left frontal lesion was markedly enhanced. (b) At the corresponding level of ADC image, the enhanced part of the lesion showed low signal in the ADC image

Table 1: Comparison of magnetic resonance DWI features between the two groups (mean±standard deviation)

Group	Case	The value of ADC ($\times 10^{-5}$ m²/s)	Confidence interval (95%)	Maximum value	Minimum value
PCNSL group	50	0.69±0.08	0.64~0.71	0.90	0.42
GBM group	50	0.78±0.03	0.74~0.79	0.90	0.67
t		8.713			
P-value		<0.05			

ADC: Apparent diffusion coefficient; GBM: Glioblastoma, PCNSL: Primary central nervous system lymphoma, DWI: Diffusion-weighted imaging

Table 2: Comparison of the difference of Ki-67 expression in patients of two groups (Cases %)

Group	Case	Ki-67			Positive rate (%)
		Negative	Slightly positive	Strongly positive	
PCNSL group	50	4 (8.0)	37 (74.0)	9 (18.0)	92.0
GBM group	50	19 (38.0)	19 (38.0)	12 (24.0)	62.0
χ^2					7.045
P-value					<0.05

GBM: Glioblastoma, PCNSL: Primary central nervous system lymphoma

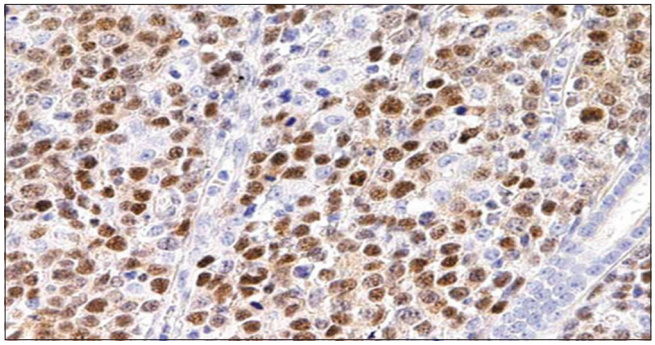

Figure 3: Positive expression of Ki-67 in primary central nervous system lymphoma tissues (Immunohistochemical staining ×200)

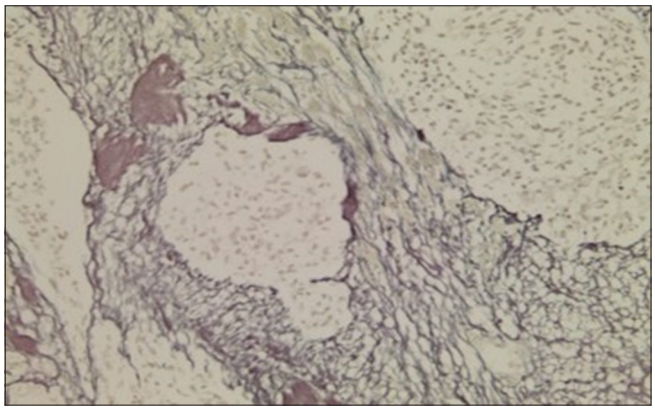

Figure 4: Positive expression of Ki-67 in glioblastoma tissues (Immunohistochemical staining ×200)

groups ($P < 0.05$). The positive rates of Ki-67 in the PCNSL group and GBM group were 92.0% and 62.0%, respectively, with significant difference between the two groups ($P < 0.05$).

According to Spearman rank correlation analysis, there was a negative correlation between the rADC value in the PCNSL group and GBM group (r PCNSL group = −0.607, $P < 0.05$); r GBM group = −0.613, $P < 0.05$). Tips: The diagnosis of intracranial PCNSL and GBM with DWI has differential value. The value of rADC is negatively correlated with Ki-67 positive rate, which provides a non-invasive quantitative index for the judgment of the deterioration degree of PCNSL and GBM.

DISCUSSION

In the past, PCNSL is considered to be idiopathic immune-deficient patients with tumors, especially human immunodeficiency virus infection, AIDS patients, and organ transplant recipients.[8,9] However, in recent years, with the intensive antiretroviral drugs usage and the intensive monitoring and early warning mechanism of high-risk groups, the incidence of PCNSL in these groups is decreasing. Now, the prevalence of immune function in normal population is increasing. PCNSL has short course of natural disease, rapid progress, poor prognosis, and highly lethal nature.[10] GBM is one of the most common brain tumors in adults, with high malignancy, rapid development, and easy to relapse.[11] There are also great differences in the treatment of the two kinds of central nervous system lesions, such as PCNSL and GBM, for example, the majority of PCNSL is treated with radiotherapy, and more of the GBM is operated on. Therefore, accurate diagnosis is of great significance for follow-up treatment.

With the continuous development of nuclear magnetic resonance technology, quantitative and qualitative analysis of tumor are also widely used in clinical diagnosis. MRI

has good resolution in cranial nervous system lesions, and it can improve the ability of differential diagnosis of intracranial nervous system diseases by multiparameter and multidirectional imaging technology. In recent years, the newly developed DWI and dynamic contrast-enhanced MRI reflect the physiological, biochemical, and pathological information of tissue by different angles. The traditional diagnosis of intracranial nervous system disease has been gradually pushed from the macro to the microlevel, which has become an important way to evaluate intracranial nervous system diseases in clinic.

In this study, we found that the MRI characteristics of PCNSL and GBM lesions were slightly different. Among them, there were differences in rADC values between the PCNSL group and GBM group, and the values of rADC in PCNSL and GBM lesions increased in turn. In the DWI scan, the volume, density, and special arrangement of the tumor cells will all affect the rADC value.[12] Patients with PCNSL have a higher density of tumor cells, abundant intracellular ribosome's, and a higher proportion of cytoplasm. In addition, the extracellular space is small, which limits the spread of water molecules in tumor cells. Therefore, the rADC value of DWI detection in PCNSL patients is relatively low. Despite the high degree of malignancy of GBM tumors, small cell gap, and limited diffusion of water molecules in tumor cells, the unit cell density of GBM patients is lower than that of PCNSL patients. Therefore, the rADC value of GBM patients is higher than that of PCNSL patients.

Nuclear-associated antigen Ki-67 is closely related to the occurrence and development of tumor, and its expression level can objectively reflect the proliferation and malignancy of tumor cells, and has become an important indicator for studying cell proliferation kinetics and evaluating biological behavior and prognosis of cancer. It has been found that the quantitative value of Ki-67 gradually increased from low-grade gliomas to GBM, and there is a significant difference between different grades of glioma.[13] The positive rate of Ki-67 expression in gliomas of various grades is different, but there are some differences. However, all of them indicate that Ki-67 is positively correlated with the pathological grading of tumor issue.[14,15] In this study, it was found that the positive expression rate of Ki-67 protein in PCNSL and GBM lesions decreased, in turn, indicating that the proliferation of tumor cells in these two lesions decreased in turn. The correlation between rADC value and Ki-67 expression rate of the two groups was compared. It was found that the rADC value of the two groups was negatively correlated with the Ki-67 expression rate, indicating that the rADC values of these two lesions can reflect the proliferation of tumor cells to a certain extent, which is closely related to the positive rate of Ki-67. The lower the rADC value is, the higher the positive rate of Ki-67 is.

Meanwhile, the proliferative activity of tumor cells is also stronger. Therefore, the ADC value can be used as an indicator to determine the intensity of Ki-67 expression and the degree of tumor proliferation.

CONCLUSION

This presenting study suggested that the diagnosis of intracranial PCNSL and GBM by DWI should have differential value; rADC value is negatively correlated with Ki-67 positive rate, which provides a non-invasive quantitative index for the judgment of the deterioration degree of PCNSL and GBM.

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