Clinical Applications of 3T MR Spectroscopy

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ABSTRACT

The purpose of this study was to assess clinical proton MR spectroscopy (MRS) as a noninvasive method for evaluating brain tumor malignancy at 3T high field system. Using 3T MRI/MRS system, localized water-suppressed single-voxel technique in patients with brain tumors was employed to evaluate spectra with peaks of N-acetyl aspartate (NAA), choline-containing compounds (Cho), creatine/phosphocreatine (Cr) and lactate. On the basis of Cr, these peak areas were quantificated as a relative ratio. The variation of metabolites measurements of the designated region in 10 normal volunteers was less than 10%. Normal ranges of NAA/Cr and Cho/Cr ratios were 1.67 ± 018 and 1.16 ± 0.15, respectively. NAA/Cr ratio of all tumor tissues was significantly lower than that of the normal tissues ($p=0.005$), but Cho/Cr ratio of all tumor tissue was significantly higher ($p=0.001$). Cho/Cr ratio of high-grade gliomas was significantly higher than that of low-grade gliomas ($P=0.001$). Except 4 menigiomas, lactate signal was observed in all tumor cases. The present study demonstrated that the neuronal degradation or loss was observed in all tumor tissues. Higher grade of brain tumors was correlated with higher Cho/Cr ratio, indicating a significant dependence of Cho levels on malignancy of gliomas. Our results suggest that clinical proton MR spectroscopy could be useful to predict tumor malignancy.

Keywords: Brain tumors, Malignancy, Magnetic resonance, spectroscopy

1. INTRODUCTION

Preliminary studies by clinical proton magnetic resonance (MR) spectroscopy indicated that proton MR spectroscopy may be able to aid in the diagnosis of various types or malignancy of brain tumors.[1-3] Because of differences in the cell type and growth characteristics of tumors, it is probable that tumor types or malignancy will have unique metabolic information. The followed studies were conducted on larger series of patients and showed the ability of clinical proton MR spectroscopy to give information on the basic metabolic processes in tumors.[4-7] However, these studies concluded that there was no reliable indicator for discriminating among tumor types or malignancy.

The aim of this study was to test the usefulness of single-voxel proton MR spectroscopy as a noninvasive method to evaluate the malignancy of brain tumors and correlation with results of surgical specimens. Our study was designed to differentiate brain tumors by using a combination of single-voxel spectroscopy, histopathological grades of malignancy in gliomas, and Ki-67 proliferating cell index (PCI) as biological malignancy index in all brain tumors.

The ability to predict the grade of malignancy of primary brain tumors, particularly gliomas is critical to clinical management decisions. The most common types in gliomas are diffuse, fibrillary astrocytomas.[8] These tumors are classified into low-grade astrocytoma (grade I/II), anaplastic astrocytoma (grade III), and glioblastoma multiforme (GM) (grade IV). There is, however, significant variation in the clinical course of these lesions and in approach to therapy. Low-grade astrocytoma is a relatively indolent lesion that is treated with surgical resection when possible, often followed by limited-field external-beam radiotherapy. Currently, there is no accepted role for chemotherapy as the primary therapy in this group of patients. Anaplastic astrocytoma is a more aggressive lesion than its low-grade counterpart. Survival in this group of patients has been shown to be significantly prolonged by the use of radiotherapy and chemotherapy.[9] GM is an aggressive glial tumor and is the most common primary tumor of the CNS, constituting approximately 20% of all primary neoplasms.[10] Most patients with glioblastoma receive radiotherapy. Younger patients

with good performance status have been shown to obtain some benefit from the addition of the chemotherapy.[11]

In the present study, we used the quantitative approach for spectral analysis by using a creatine (Cr) reference. Thus, the present method showed that clinical proton MR spectroscopy could provide information to help discriminate among brain tumor grades of malignancy.

2. MATERIALS AND METHODS

Subjects

During the period from January 2000 to June 2002, ten normal volunteers (5 males and 5 females) aged 19-41 years (median, 33.5 years) and 23 patients (8 males and 15 females) aged 11-73 years (median, 47.8 years) with brain tumors (12 gliomas, 8 meningiomas and 3 others) were examined by localized *in vivo* ^{1}H MR spectroscopy before any treatment. Tumor types and pathologic findings are listed in Table 1. All the patients underwent surgery to remove the tumors after the examination. The complete histological diagnosis of each tumor was provided by the clinical pathologists. Tumors were included in the study only when we could locate at least 2.5 mL of volume of interest (VOI) within the tumor body while avoiding the inclusion of macroscopic cysts and necrosis in the VOI. The malignancy of the tumors was evaluated with Ki-67 PCI and the histopathological grades of malignancy for gliomas were classified from grade I to IV according to the World Health Organization grading.[12] After complete description of the study to the subjects, written informed consent was obtained from each subject.

Normal control subjects were recruited from the Catholic University Medical Center (CUMC) staff, residents, interns, and graduate students. The volunteers were screened for medical and neurological illness and history of substance abuse. None of normal control subjects had a history of substance dependence or current abuse or a history of neurological disorders.

^{1}H MR Spectroscopy

In vivo ^{1}H MRS examinations were performed on a Magnus 2.1 for Magnum 3 tesla MRI/MRS system (Medinus Co., LTD. Seoul, Korea) with a standard quadrature birdcage head coil. Localized single voxels (7-8ml) centered on the volume of interested lesion in patients with brain tumors selected using the T2-weighted MR images (TR 2500 ms; TE 90 ms) with fast spin echo (FSE) with the echo train length of 8. A stimulated-echo acquisition mode (STEAM)[13,14] was used as the localization method in this study. Suppression of the water signal was performed by using a three-pulse chemical shift selective sequence (CHESS).[15,16] Offsets of the higher order and linear shim coils were adjusted by the auto prescan (APS) for optimization of the homogeneities of the total and the localized volumes of the brain, respectively. The strength of the transmitter RF power, the receiver gains, and the three RF pulses for suppression of the water signal were also adjusted by the APS. After APS, typical line width (full width at half maximum; FWHM) was usually 2 to 4 Hz, which gave 97-99% of the suppression factor. Image guided STEAM spectra were obtained with a TE of 20 msec, TR of 2000 msec, data points of 2048, spectral bandwidth of 2500 Hz, and acquisition averages of 128.

Processing

Raw data acquired were transferred to an independent console and processed by MagSpec data analysis package (Medinus Co., LTD. Seoul, Korea). The postprocessing consisted of removal of residual water signal, correction of the heavy eddy current if needed, Lorenz-to-Gauss transformation, Gaussian line broadening of 0.5Hz, zerofilling of 4K, Fourier transformation, and zero order phasing of the transformed spectrum. Any resulting spectra which contained the features of inadequate signal-to-noise ratio (SNR), outer volume contamination, distorted baselines, severe phase distortion due to the heavy eddy current, hardware artifacts, or inappropriate suppression of the water signal were regarded unacceptable, and were not included in data analysis. Peak areas were obtained from the spectra by employing the Marquardt algorithm to fit a Lorentzian type.[17] Resonance peak assignments of major neurometabolites at *in vivo* ^{1}H MRS were NAA at 2.02 ppm, Cr at 3.00 ppm, and Cho at 3.20 ppm. The relative ratios of NAA to Cho, NAA to Cr and Cho to Cr were calculated in the lesion of all patients with brain tumors. Alterations of metabolite ratios were blindly evaluated and finally compared with the pathological findings in patients with brain tumors. The lactate assignment was made by the formation of the characteristic doublet caused by the J coupling and/or inversion of the peak at the echo time of 136 milliseconds caused by the J modulation.[18]

Statistics

Statistical analysis was performed using statistical software (SPSS for Windows, Version 6.0, SPSS, Chicago, IL U.S.A). The spectroscopic data were analyzed with a paired-sample Students t-test, where $p < 0.05$ was considered significant to account for multiple comparisons.

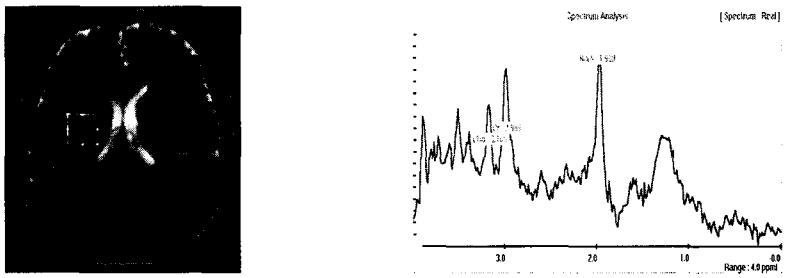
3. RESULTS

Figure 1 shows the VOI in the temporoparietal white matter and the acquired spectrum in a typical normal volunteer. The variation of metabolites measurements of the designated region in 10 normal volunteers was less than 10%. Normal ranges of NAA/Cr and Cho/Cr were 1.67±018 and 1.16±0.15, respectively.

As can be seen in Table 1, twelve gliomas included 1 grade I tumor (pilocytic astrocytoma), 2 grade II tumors (astrocytomas), 4 grade III tumors (2 anaplastic astrocytomas, 1 anaplastic oligodendroglioma and 1 anaplastic mixed glioma), and 5 grade IV tumors (5 glioblastomas), 1 medulloblastoma, 1 neurocytoma and 1 lymphoma. Pathologic diagnoses of 8 diagnosed meningiomas indicated that 3 were meningotheliomatous, 2 were anaplastic, 1 was secretory, 1 was transitional, and 1 was fibrous. None was malignant at histologic examination.

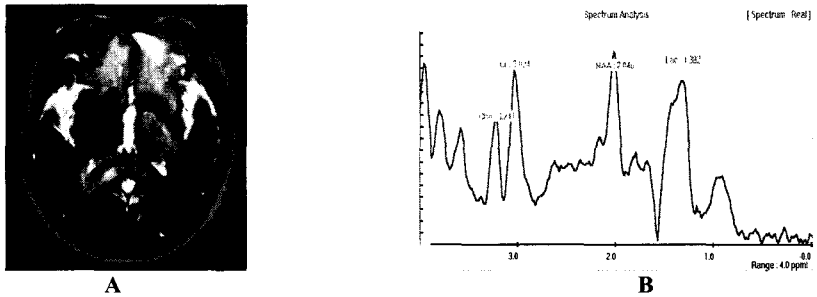
The representative cases are presented in Figures 2 through 6. High-grade gliomas showed elevated Cho/Cr ratio, while low-grade gliomas and meningimas had Cho/Cr values within the normal ranges (Table 1). All tumor cases showed the decreased NAA/Cr ratio. The decrease in the NAA peak was the actual cause of the low NAA/Cr ratio. However, NAA/Cr ratio was not correlated with malignancy.

Statistically significant differences between low-grade gliomas (grades I and II) and high-grade gliomas (grades III and IV) were shown in the levels of Cho/Cr and NAA/Cr (p=0.001). Lactate was more or less present in all the gliomas. Although high-grade gliomas tended to have higher lactate values than did low-grade gliomas, no statistically significant difference between malignancies was detected. With meningiomas in general, the NAA/Cr values were much lower than normal. NAA signal was not observed in the one case (patient #18). Also, lactate was not observed in 4 cases among 8 cases.



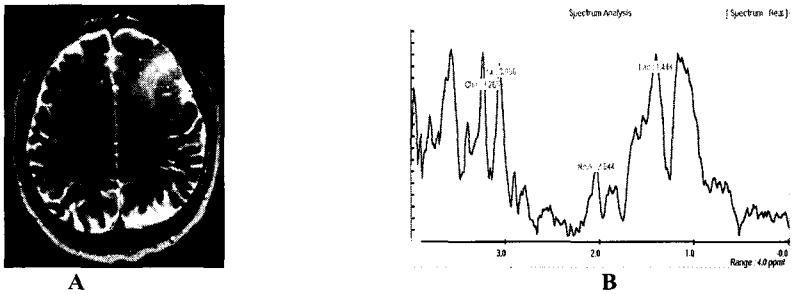
A B

Figure 1. A. Typical T2-weighted axial MR image in normal volunteer. B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).



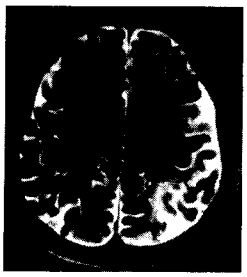
A B

Figure 2. A. T2-weighted axial MR image of low-grade glioma, pilocytic astrocytoma (grade I, patient #1). B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).

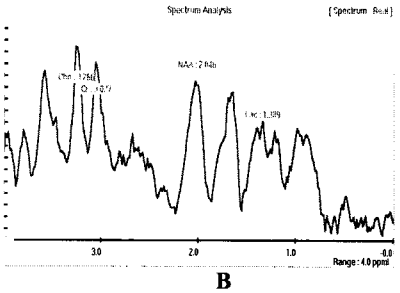


A B

Figure 3. A. T2-weighted axial MR image of low-grade glioma, astrocytoma (grade II, patient #3). B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).



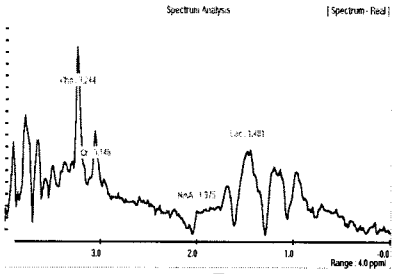
A



B

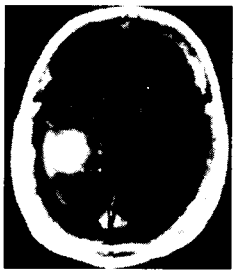
Figure 4. A. T2-weighted axial MR image of high-grade glioma, anaplastic astrooligodendroglioma (grade III, patient #7). B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).

A

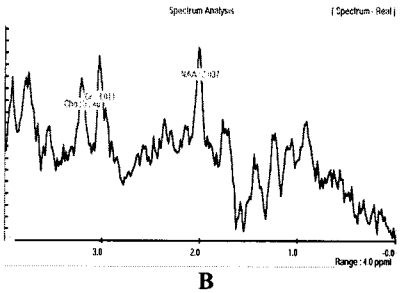


B

Figure 5. A. T2-weighted axial MR image of high-grade glioma, glioblastoma (grade IV, patient #10). B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).

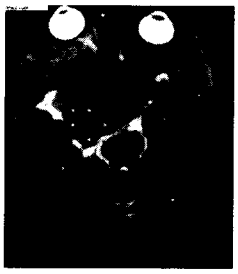


A

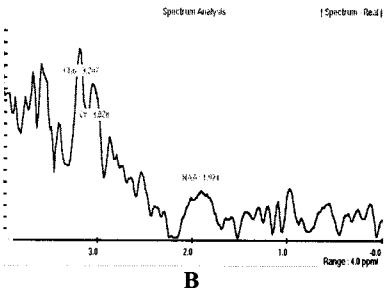


B

Figure 6. A. T2-weighted axial MR image of menigioma (patient #16). B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).



A



B

Figure 7. A. T2-weighted axial MR image of menigioma (patient #22). B. ^{1}H MR spectrum obtained from VOI shown in A, acquired with STEAM. Chemical shifts are indicated in parts per million (ppm).

Table 1

Patient#	Age/Sex	Tumor Type and Location	Pathologic FInding	Lesion		
				Cho/Cr	NAA/Cr	Lac/Cr
1	F/49	Pilocytic astrocytoma, Lt. Frontal	KI-67 index:5-10%, GFAP:+	0.44	0.26	1.65
2	F/42	Astocytoma, Lt. Temporal	Ki-67 index:less than1%	0.45	0.80	1.50
3	M/45	Astocytoma, fibrillary type, low-grade, Lt. Fontal	Ki-67 index:less than1%, GFAP: +	0.91	0.67	1.30
4	F/28	Anaplastic astrocytoma	Ki-67 index:27%	1.10	0.46	1.87
5	F/73	Anaplastic astrocytoma	Ki-67 index:2-3%	1.76	0.94	1.30
6	F/29	Anaplastic oligodendroglioma, Rt. Frontal	Ki-67 index:27%	1.85	1.00	4.00
7	F/49	Anaplastic mixed astrooligodendroglioma, Lt. Temporal	Ki-67 index:8%, GFAP:+	1.99	0.43	1.27
8	F/55	Glioblastoma, Rt. Parietal	Ki-67 index:40%	2.02	0.32	3.80
9	F/64	Glioblastoma, Rt. Temporoperiatal	GFAP:+	2.10	0.11	9.10
10	M/46	Glioblastoma, Rt. Frontal	Ki-67 index:7-8%	2.11	1.17	4.95
11	M/11	Medulloblastoma, Cbll.	Ki-67 index:45-50%, GFAP:focally +, Neurofilament:+	2.26	0.99	2.75
12	M/59	Glioblastoma, Rt. Temporal	Ki-67 index:30%	2.28	0.52	4.25
13	F/25	Central neurocytoma, 3rd ventricle	Vimentin:-, NSE:+, GFAP:-, Synaptophsin:+	2.35	1.29	2.50
14	M/63	Peripheral T-cell lymphoma, Both temporal	LCA:+, UCHL-1:-, L26:-, GFAP:-	3.47	1.48	2.62
15	M/64	Glioblastoma, Rt. Temporal	Ki-67 index:5%	3.80	0.90	5.58
Mean ± SD				1.93± 0.95	0.69± 0.41	3.23± 2.15
16	M/57	Meningioma, menigotheliomatous type, Rt. Temporal	Ki-67 index:less than 1%	0.50	1.19	*
17	F/61	Anaplastic meningioma, Lt. Falx	Ki-67 index:1-2%	0.51	1.21	5.00
18	F/42	Anaplastic meningioma, Rt. CPA	Ki-67 index:less than 1%	0.60	*	*
19	F/59	Meningioma, secretory type, Rt. CPA	Ki-67 index:1%	0.61	0.25	4.62
20	M/66	Meningioma, menigotheliomatous type, Rt. Frontal	Ki-67 index:5-6%	0.93	1.17	*
21	F/26	Meningioma, transitional type, Rt. Parietal	Ki-67 index:less than 1%	1.34	0.59	3.60
22	F/44	Meningioma, meningotheliomatous type, Rt. CPA	Ki-67 index:1-2%	1.61	0.67	*
23	F/43	Meningioma, fibrous type, Rt. Parietal	Ki-67 index:less than 1%	1.69	0.20	2.50
Mean ± SD				0.97± 0.50	0.66± 0.49	1.97± 1.12

*: Not observed

Note. Ratios are mean±SD (standard deviation).

4. DISCUSSION

The capability of a non-invasive technique, graphically localized, water-suppressed *in vivo* ^{1}H MRS at 3 tesla has been demonstrated to monitor metabolic levels of brain tissue in tumor patients. The present study shows that *in vivo* ^{1}H MR spectral patterns of tumors differed from those of normal brain tissue (Figures 1-6). The ratios Cho/Cr and NAA/Cr in tumor spectra were significantly different from normal control spectra in Table 1 (p=0.001). Since the signal intensities of NAA and Cho are responded sensitively in the neoplastic tissue, the ratios of NAA/Cr and Cho/Cr in gliomas are substantially changed comparing with normal tissue.

In the present study, the Cho/Cr ratio of high-grade gliomas was significantly higher than that of low-grade gliomas. This result suggested a significant dependence of Cho levels on malignancy of gliomas. Our study is in good agreement with *in vitro* MRS study that can indicate the types of brain tumors and the degree of malignancy by showing the changes in metabolite concentrations.[19] For example, high-grade astrocytic tumors had lower NAA

concentration and higher Cho concentration than did low-grade astrocytomas. The Cho signal originates mainly from intermediates of phospholipid metabolism such as phosphocholine and glycerophosphorylcholine. Cho is a precursor for the biosynthesis of membrane lipids. All tumor spectra show an increased Cho signal. This may reflect an elevated concentration of mobile precusors participating in cell membrane turnover during cell proliferation.

The predominant signal at 2.02 ppm in potentially normal brain tissue is that of the CH_3 resonance of NAA. This compound is located almost exclusively in neurons, and believed to be a neuronal marker for the presence of intact neurons.[20] The decrease of NAA concentration in tumor tissue indicates the neuronal loss or degeneration itself, and a decrease or displacement of neurons by the tumor. An accumulation of lactate usually signifies a disturbance of normal energy metabolism due to incomplete oxidation of pyruvate. In our present results, the reduction of NAA/Cr ratio occurred most prominently in the brain tumor patients.

We carefully included only the tumor body and not the surrounding brain, cysts, or necrosis. Therefore, most of the spectral changes observed in the present study are attributable to changes in the tumor metabolism. Two characteristic features of meningiomas were that NAA was absent and that Cho was not above normal levels. However, the NAA signal was observed in several meningiomas, which should not contain NAA, so the spectra may have suffered from some partial volume effects.[21] In some cases, the unavoidable inclusion of microcysts or micronecrosis may also have caused lactate and aliphatic signals. In single-voxel spectroscopy, the voxel placement is critical to the examination. For this reason, we chose to undertake spectroscopy after a contrast-enhanced MR imaging series had been obtained, as this allows for precise voxel placement over an enhancing region so that signal is acquired only from viable tumor tissue.[22,23] Therefore, in the present study we strictly limited the VOI to be within the tumor body, and our results indicated that the malignancy of the gliomas can be predicted to a fairly large extent.

Previous proton MR spectroscopy studies on human brain tumors can be divided, in terms of methodology, into localized single-voxel and the chemical-shift imaging techniques. Spectral interpretations were made by calculation of either metabolite ratios (eg, NAA/Cr, NAA/Cho) or ratios to apparently normal regions (eg, tumor NAA/contralateral brain NAA). Using the single-voxel method, Kugel and coworkers[24] found a clear difference in spectra between gliomas and meningiomas. This is illustrated that the relative ratios of proton metabolites in tumor tissue directly relates with the diagnosis of the malignancy. Since *in vivo* ^{1}H MRS provides the numbering ratios for diagnosis of tumor grade, the interpretation for tumor grade could be helpful. Moreover, the numbering system suggests that the possibility of subjective misreading and misinterpretation may be minimized and eventually excluded.

In the present study, the lactate/Cr value tended to be higher in grade III and IV gliomas than in grade I and II gliomas; however, as shown in Table 1, the lactate/Cr value was not a reliable indicator of malignancy. Data from several studies agree with our results showing the varying degrees of lactate across malignancies.[25,26] The reason for this variation may be that lactate arises not only from the tumor itself but also from necrosis or cysts within the tumor. Our results also showed that benign meningiomas had low Cho levels. Ott and coworkers[6] reported that malignant meningiomas had higher Cho/Cr ratios than did benign meningiomas. It will be valuable to investigate further whether Cho levels obtained by proton MR spectroscopy can predict the oncologic activity of meningiomas that are histologically indistinct.

In conclusion, significant differences between tumor and normal tissue was observed in the spectral pattern of image-guided water-suppressed *in vivo* ^{1}H MRS at 3 tesla system. Proton MR spectroscopy at 3T MR system was tested for its validity and limitations in the evaluation of malignancy of gliomas and meningiomas. Histologic gradings of glioma were, to a great extent, predictable by Cho/Cr value. In our 3T MRI/MRS facility, the spectral differences in various tumor types are keeping in data bank for further detail analysis and interpretation, eventually for the accurate and sensitive diagnosis. A method that could precisely assign observed lesions to specific diagnostic gradings could reduce the need for surgical biopsy and thus reduce patient morbidity and mortality. The present results indicate the usefulness of proton MR spectroscopy in the evaluation of tumor malignancy noninvasively. Therefore, we suggest that *in vivo* ^{1}H MRS can improve the high quality of diagnostic level in terms of biochemical and metabolic process in neoplastic tissue.

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