

MAJOR PAPER

The Significance of Lactate and Lipid Peaks for Predicting Primary Neuroepithelial Tumor Grade with Proton MR Spectroscopy

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Purpose: ¹H-MRS is a non-invasive technique used to assess the metabolic activity of brain tumors. The technique is useful for the preoperative prediction of tumor grade, which is important for treatment planning and accurate prognosis. We used ¹H-MRS to study the lactate peak, which appears in various conditions, including hyperglycemia, ischemia, and hypoxia and lipid peak, which is associated with necrotic cells. The purpose of this study was to retrospectively examine the frequency and significance of lactate and lipid peaks in relation to brain tumor grade.

Materials and Methods: Fifty-five patients diagnosed with neuroepithelial tumors of Grades I (3 cases), II (11 cases), III (15 cases), and IV (26 cases) were enrolled. Biopsies were excluded. Single voxel (TE = 144 ms) point resolved ¹H-MRS spectroscopy sequences were retrospectively analyzed. An inverted doublet peak at 1.3 ppm was defined as lactate, a negative and positive peak was defined as combined lactate and lipid, and a clear upward peak was defined as lipid.

Results: Lactate peaks were detected in all grades of brain tumors and were least common in Grade II tumors (9.1%). The frequency of combined lactate-lipid peaks was 0% (Grades I and II), 8.3% (Grade III), and 44% (Grade IV). Grade IV tumors were significantly different to the other grades. There were three cases with a lipid peak. All were glioblastoma.

Conclusions: The presence of a lac peak may be useful to largely rule out the Grade II tumors, and allow the subsequent differentiation of Grade I tumors from Grade III or IV tumors by conventional imaging. The presence of a lipid peak may be associated with Grade IV tumors.

Keywords: *lactate, lipid, magnetic resonance spectroscopy, brain tumor, glioma*

Introduction

Lactate (Lac) production is considered to be an indicator of altered metabolism in brain tumors. Glucose metabolism is the sole mechanism by which the brain generates energy in the form of adenosine triphosphate (ATP). In the normal brain, glucose is oxidized completely to CO₂ and H₂O through the tricarboxylic acid (TCA) cycle and electron transport system to generate 36 mol ATP. However, if O₂ is insufficient, the end product of glycolysis, pyruvate, cannot enter the TCA cycle and is converted

to lactate generating 2 mol ATP. This lac producing pathway is referred to as “anaerobic glycolysis”, and results from a mismatch between glycolysis and oxygen supply.¹ Lactate accumulation in brain tumors is caused by increased glycolysis, and is associated with ischemic change in the poorly perfused tumor parenchyma,² or an increased incidence of necrotic tissue.³

Lipids (Lip) exist as a macromolecule in cell membranes and myelin sheaths in normal brain tissue. When brain tissue is damaged or disrupted as occurs during brain tumor growth, the lip macromolecules are transformed into mobile lip.⁴

¹H-MRS is a noninvasive approach for the evaluation of metabolic patterns in human brain tumors, and is useful for detecting lac and lip peaks. Lip can be detected by magnetic resonance spectroscopy (MRS) in high-grade tumors and is correlated with the amount of microscopic cellular necrosis.⁴

Several studies suggested that high-grade gliomas can be distinguished from low-grade tumors by higher lac peaks,^{5,6} although other researchers did not find a significant correlation.^{7,8}

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The purpose of this study was to retrospectively examine the correlation of lac and lip peak frequency with tumor grade, and evaluate the utility of lac and lip peak frequency for predicting tumor grade.

Materials and Methods

Study subjects

Fifty-five patients diagnosed with neuroepithelial tumors were enrolled from January 2007 to March 2013 at St. Marianna University of Hospital, Kawasaki, Japan. The patients included 34 men and 21 women, ranging in age from 26 to 73. Biopsies and recurrent cases were excluded. Histologic analyses was performed, according to the World Health Organization brain tumor classification revised in 2007.⁹ Three patients were diagnosed with Grade I tumors, 11 with Grade II tumors, 15 with Grade III tumors, and 26 with Grade IV tumors. Of the Grade I tumors, two were classified as pilocytic astrocytoma and one as dysplastic gangliocytoma. The Grade II tumors included four oligoastrocytoma, five diffuse astrocytoma, one ependymoma, and one pleomorphic xanthoastrocytoma. Of the Grade III tumors, 10 were anaplastic astrocytoma, four anaplastic oligoastrocytoma, and one anaplastic oligodendroglioma. The Grade IV tumors included 25 glioblastoma and one medulloblastoma.

Proton MRS

MR imaging was performed on all of the study patients with a 1.5T MR system (Achieva; Philips Healthcare, Best, The Netherlands). In all cases, single voxel ¹H-MRS was performed with the same MR unit using a point resolved spectroscopy sequence (TR, 2000 ms; TE, 144 ms; average 128). Tumor regions of interest were identified on the basis of abnormal signal intensities seen on T₂-weighted images (T₂WI), fluid attenuated inversion recovery (FLAIR) and the enhancing portion of lesions seen on contrast-enhanced T₁-weighted images (T₁WI) after intravenous injection of 0.1 mmol/kg gadoterate meglumine (Dotarem; Guerbet, Paris, France). The voxel dimensions were 1.5 × 1.5 × 1.5 cm (total volume of 3.375 cm³). Experienced neuroradiologists (H. N., T. S.) carefully positioned the voxels to include the largest enhancing region and to avoid contamination by adjacent normal tissue, the skull base, any hemorrhagic or calcified regions, and the ventricular system. For unenhanced tumors, the volume of interest (VOI) was placed as carefully as possible to exclude the fluid portion.

The metabolites were assigned as choline (Cho) at 3.22 ppm, creatine (Cr) at 3.02 ppm, N-acetylaspartate (NAA) at 2.02 ppm, lipid at 0.9 to 1.3 ppm, and lactate at 1.33 ppm. A lactate peak was identified as an inverted doublet peak at TE = 144 ms. A negative peak in the left component, occurring with a decreased negative peak in the right component, and a positive peak at 1.3 ppm, was defined as combined lac

Table 1. Frequency of lactate peaks

Grade	Cases	Frequency (%)
1	3/3	100.0
2	1/11	9.1
3	12/15	80.0
4	18/26	69.2

and lip (lac/lip). Clear upward peaks at 1.3 and 0.9 were defined as lipid.

For the quantitative evaluation of lac peaks, excluding the lac/lip peak cases, the absolute height of the lac/normal control Cr ratio was used. The control Cr represented an area in the contralateral side, or a remote section of the lesion. A neuropathologist (M.D.) confirmed that the operative area corresponded with the VOI.

Ethical approval for this study (# 2491) was obtained from the Institutional Review Board of St. Marianna University.

Statistical analysis

The frequency of lac and lip peaks relative to tumor grade was evaluated by the Ryman method. The frequency of lac/lip combined peaks in Grade III and IV tumors was evaluated by the Fisher's exact test. Kruskal-Wallis tests were used for quantitative evaluation, and $P < 0.05$ was considered significant.

Results

Frequency of lac peaks

The frequency of lac peaks relative to tumor grade is shown in Table 1. Lac peaks were significantly associated with Grade I (Fig. 1), Grade III, and Grade IV tumors. Remarkably, all Grade I tumors had lac peaks. Conversely, only one of 11 Grade II tumors had a lac peak. Statistically, the frequency of lac peaks was significantly different across tumor grades ($P < 0.05$).

Frequency of lac/lip combined peaks

One lac/lip combined peak was observed among 12 lac peak positive Grade III tumors (Table 2). Among 18 lac positive Grade IV tumors, eight cases exhibited lac/lip combined peaks. One example is described in Fig. 2. Lac/lip combined peaks were significantly more frequent in Grade IV tumors compared to Grade III tumors ($P = 0.04$).

Frequency of lip peaks

There were three clear lipid peak tumors and all were glioblastoma. One example is described in Fig. 3.

Quantitative evaluation of lac peaks

No significant differences were found between Grade I, III, or IV tumors.

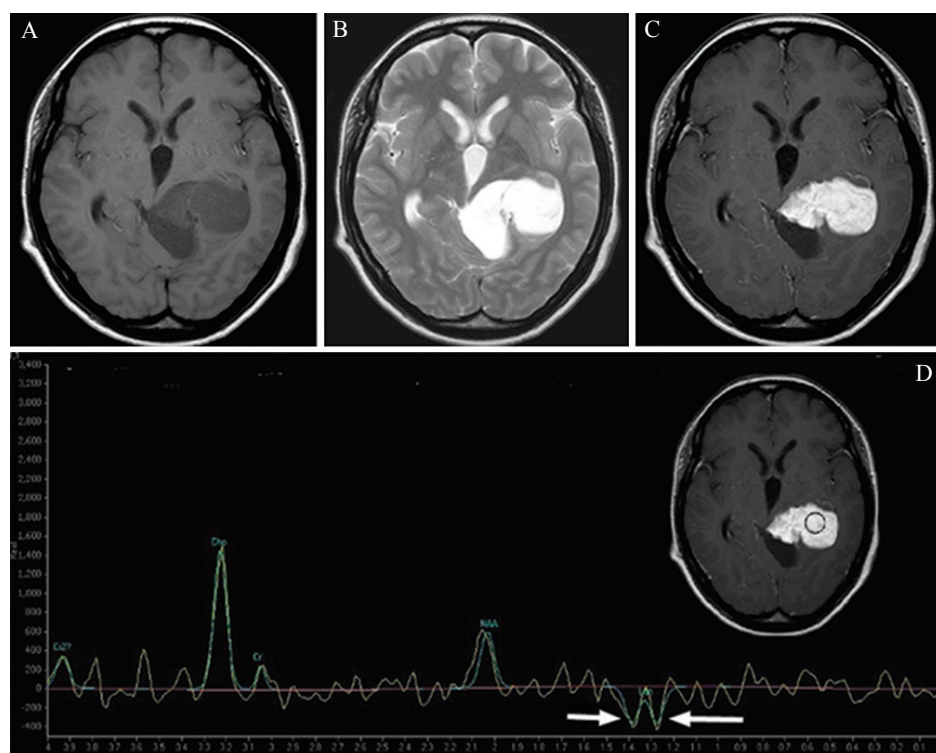


Fig. 1 Pilocytic astrocytoma in a 33-year-old woman. An oval shaped mass is located in the left temporal lobe abutting the left lateral ventricle with low signal intensity on T_1 -weighted images (T_1 WI) (A) and hyperintensity on T_2 -weighted images (T_2 WI) (B). Homogeneous enhancement is observed on Gd T_1 WI (C). Magnetic resonance spectroscopy (MRS) demonstrates increased choline (Cho) peak and decreased N-acetylaspartate (NAA) (D). Arrows indicate doublet peak representing a lac peak.

Table 2. Lac/lip peak frequency among lactate-peak positive cases

Grade	Cases	Frequency (%)
1	0/3	0
2	0/1	0
3	1/12	8.3
4	8/18	44.4

Lac/lip, Lactate and lipid combined.

Discussion

Grading neuroepithelial tumors accurately have significant implications for the management of patients because low and high-grade tumors are treated differently. For low-grade tumors, resection is usually undertaken at the time of diagnosis. The potential benefits of this approach include more accurate diagnosis, rapid palliation of symptoms, extension of survival, reduced chance of malignant transformation, and cure.¹⁰ The utility of adjuvant therapy, such as chemotherapy or radiotherapy, is not well established.^{11,12} On the other hand, the utility of radiotherapy and chemotherapy after surgery is well established for patients diagnosed with high-grade tumors,^{13,14} and if considered safe, surgery for maximal tumor resection extends survival when compared to biopsy, subtotal or partial resection.¹⁰

Preoperative tumor grading is also important for accurate prognosis, which varies according to tumor grade. Five-year survival rates for Grades I, II, III, and IV brain tumors are 94%, 51.6%, 25.2%, and 8.9%, respectively.^{15,16} Grading

from conventional MR imaging is sometimes unreliable. One study demonstrated contrast enhancement in 66.7%, 75.0%, and 100% of grade II, III, and IV gliomas, respectively, which suggests it is difficult to grade gliomas precisely based on structural imaging.¹⁷ ^1H -MRS is a noninvasive technique that is receiving increased attention for the diagnosis of brain tumors before surgery, and may assist with tumor grading.¹⁸ The technique provides additional information on metabolic activity in particular areas of the tumor, and can measure various metabolic indicators *in vivo*, such as Cho, Cr, NAA and pathologic levels of lac and lip. The non-invasive assessment of a combination of these parameters has been reported to increase the diagnostic accuracy of glioma grading.¹⁹ There is extensive literature demonstrating the metabolic ratios of Cho/Cr, NAA/Cr, mlns/Cr to be useful in grading tumors and predicting malignancy. The sensitivities of Cho/Cr and Cho/NAA were 97.5% and 96.7%, respectively, in this study confirming that metabolite ratios can be useful for determining tumor grade. However, low specificities were noted due to the high levels of Cho that were observed in low-grade gliomas.²⁰

Although a lac peak is absent in healthy brain tissue, it is produced under conditions of anaerobic glycolysis commonly associated with pathological conditions such as hypoxia and hyperglycemia.⁶ High lac concentrations have been reported in both benign and malignant brain tumors, suggesting that the condition is independent of tumor grade.³ Consistent with these data, we detected lac peaks in Grade I, III and IV brain tumors. However, we hypothesize that the etiology of benign and malignant brain tumors is different

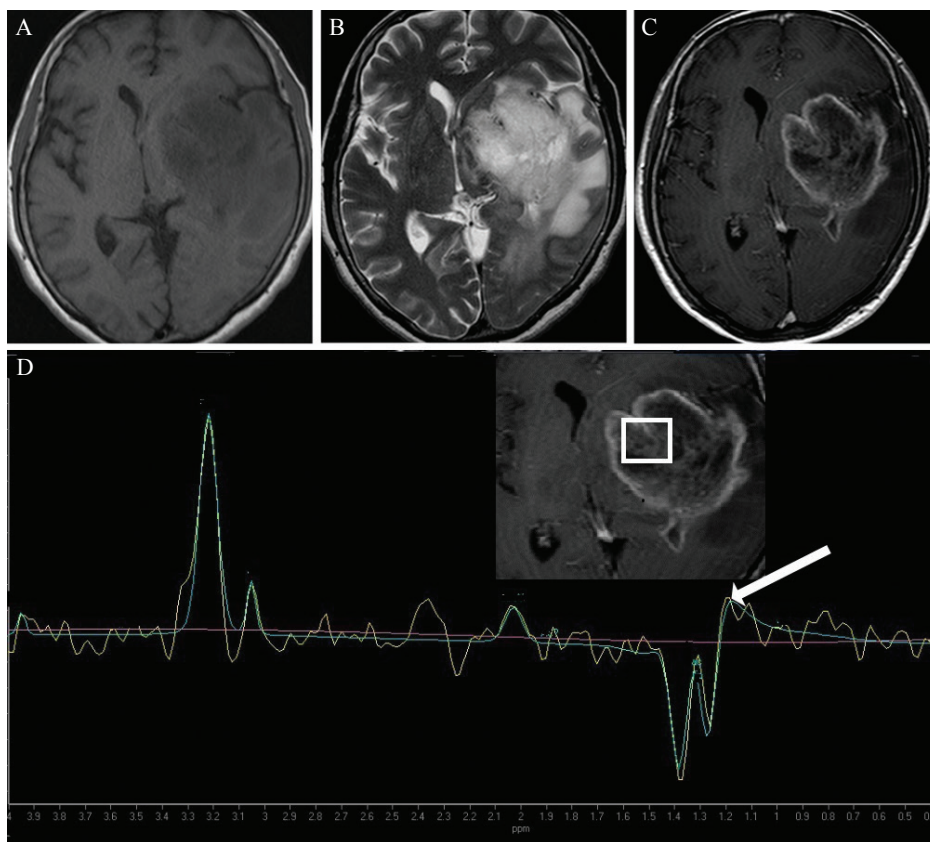


Fig. 2 Glioblastoma in a 69-year-old woman. (A) Axial T₁-weighted images (T₁WI) shows an irregular low signal intensity mass mainly located in the left basal ganglia. (B) Axial T₂-weighted images (T₂WI) shows a heterogeneous signal mass extending into the temporal lobe. Due to the mass effect, the left ventricle is obliterated. (C) Axial Gd T₁WI shows heterogeneous enhancement with a thick irregular wall. The mass consists of linear, nodular enhancement areas and a no enhancement area suggesting cyst or necrosis. (D) Magnetic resonance spectroscopy (MRS) shows lip peak (arrow) as well as a lac peak which is defined as “lac and lip (lac/lip) combined”.

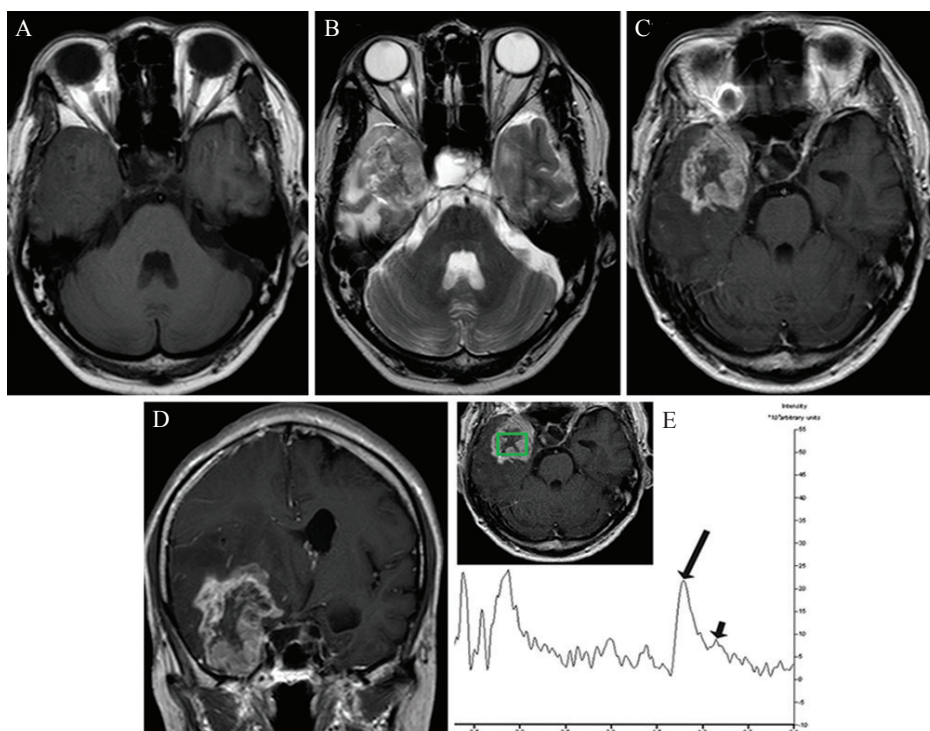


Fig. 3 Glioblastoma in a 56-year old man. (A) Axial T₁-weighted images (T₁WI) shows a low intensity mass in the lower temporal lobe. (B) Axial T₂-weighted images (T₂WI) shows heterogeneous intensity that consists of intermediate signal intensity and high intensity at the central portion. (C) Axial Gd and coronal Gd T₁WI shows heterogeneous enhancement with a thick irregular wall. (D) T₁WI shows heterogeneous enhancement with a thick irregular wall. (E) Magnetic resonance spectroscopy (MRS) (TE = 144 ms) shows a prominent lip peak (long arrow indicates the peak at 1.3 and short arrow indicates the peak at 0.9 ppm).

with Grade I tumors characterized by hypermetabolic glucose consumption, and Grade III and IV tumors producing lactate under hypoxic conditions and/or in association with an increased incidence of necrotic tissue.

Our results showed that among lac positive tumors that also exhibited lip peaks, a Grade IV classification was more likely, and a clear lipid peak was associated with glioblastoma (Grade IV tumors). Our data linking lip peaks with Grade IV tumors are consistent with earlier reports of high lip peaks in glioblastoma, which has been ascribed to an increased incidence of necrotic regions in these highly aggressive tumors.²¹

Our study is to classify the grading (from Grade I to Grade IV) of the neuroepithelial tumor. These include glioma and non-glioma (medulloblastoma and ependymoma). Medulloblastoma and ependymoma show no lactate nor lipid peak according to the previous study which was applied short and long TE.²² If we had focused on glioma, we would have different results. We would like to know the significance of the primary neuroepithelial tumor throughout Grade I to Grade IV.

When considering ¹H-MRS as a diagnostic tool for neuroepithelial tumors, the presence of a lac peak may be useful to largely rule out the presence of Grade II tumors, and allow the subsequent differentiation of Grade I tumors from Grade III or IV tumors by conventional imaging. Grade IV tumors can then be diagnosed based on the presence of a lip peak. When we see the malignant tumor, we think as a rule lac is associated with grade III, while lipid is associate with grade IV. It is important that we can clearly demonstrate the significance of lac and lipid separately, although lac and lipid cannot be precisely differentiated using the medium echo time (TE = 144 ms) applied in this study.

¹H-MRS may be very useful for monitoring benign glioma that present initially without a lac peak. The later appearance of a lac peak may signify malignant transformation (Grade II to Grade III), prompting more aggressive intervention.

There are a number of limitations to our study. Firstly, above mentioned, all the spectra were based on a medium echo time (TE = 144 ms), which is potentially problematic for the differentiation of lactate and lipid peaks, which have similar locations at 1.3 ppm. Only clear downward peaks were defined as lac peaks, and the presence of upward and downward peaks was defined as lac and lipid combinations. A more precise differentiation of lac and lipid contributions might be attained if several proton MRS examinations with different echo times (TE = 35, 288 ms) were used. However, such an approach would significantly increase the investigation time and be impractical in a clinical setting. It is possible that because we regarded only downward peaks as lac peaks, we have underestimated lac peak frequency as a result of some lac peaks being masked by positive lipid peaks.

Secondly, the retrospective nature of our study should also be considered as a limitation.

Finally, our study was not classified as histological type, but only as classified as grading. If the histologic type was unformed, we would have different results.

Conclusions

Our data suggest that the presence of a lac peak is a clear predictor of a Grade I, II, or IV brain tumor, while diagnosis of a Grade II tumor is less likely. It is important to consider conventional imaging for the differentiation of Grade I from Grade III or IV tumors. The presence of lac/lipid combination peaks or clear lip peaks is most consistent with Grade IV tumors.

Conflicts of Interest

The authors does not have any conflicts of interest to declare.

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