Comparative Value of 2-Hydroxyglutarate–to–Lipid and Lactate Ratio versus 2-Hydroxyglutarate Concentration on MR Spectroscopic Images for Predicting Isocitrate Dehydrogenase Mutation Status in Gliomas

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Supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology (grant number: NRF-2017R1A2A2A05001217).

Conflicts of interest are listed at the end of this article.

Radiology: Imaging Cancer 2020; 2(4):e190083 • https://doi.org/10.1148/rycanc.2020190083 • Content codes: [MR] [NR] [OI]

Purpose: To compare the ability of 2-hydroxyglutarate (2HG)–to–lipid and lactate (2HG/(lipid + lactate)) ratio with the ability of 2HG concentration alone to predict the isocitrate dehydrogenase (IDH) mutation status in patients with glioma.

Materials and Methods: In this retrospective study, consecutive patients with histopathologically proven glioma were enrolled between July 2016 and February 2019. A total of 79 patients were enrolled (mean age, 44 years; 49 men). The 2HG concentration and other MR spectroscopic parameters were measured by single-voxel point-resolved spectroscopy before surgery. The diagnostic performance of the 2HG concentration and 2HG/(lipid + lactate) ratio were calculated. Internal validation was assessed by the bootstrap approach with 1000 bootstrap resamples. Differences in the predictive accuracy of 2HG/(lipid + lactate) ratio and 2HG concentration were determined by calculating the integrated discrimination improvement. The diagnostic accuracy (sensitivity, specificity, and area under the receiver operating characteristic curve [AUC]) of these measures was also compared separately in patients with glioblastomas and patients with lower-grade gliomas.

Results: Of the 79 enrolled patients, 28 had IDH mutations and 51 had wild-type IDH. The sensitivity, specificity, and AUC of 2HG concentration for predicting IDH-mutant gliomas were 89% (25 of 28), 67% (34 of 51), and 0.80 (95% confidence interval [CI]: 0.70, 0.88; C statistic, 0.80), respectively. The sensitivity, specificity, and AUC of the 2HG/(lipid + lactate) ratio for predicting IDH-mutant gliomas were 79% (22 of 28), 92% (47 of 51), and 0.90 (95% CI: 0.81, 0.96; C statistics, 0.90), respectively. The optimal cutoff value for the 2HG/(lipid + lactate) ratio was 0.63. The 2HG/(lipid + lactate) ratio was significantly better for predicting IDH-mutation status than the 2HG concentration alone (P = .052). In lower-grade glioma, the 2HG/(lipid + lactate) ratio and the 2HG concentration showed comparable diagnostic performance (P = .72).

Conclusion: The 2HG/(lipid + lactate) ratio is more accurate for predicting IDH mutation status in patients with glioma than the 2HG concentration alone.

The use of 2-hydroxyglutarate (2HG) MR spectroscopy is accurate in predicting isocitrate dehydrogenase (IDH) mutation status in patients with glioma, especially those with lower-grade (World Health Organization [WHO] grades 2 and 3) glioma, with a sensitivity of 95% and a specificity of 91% (1,2). Because 2HG is an oncometabolite that appears as a direct consequence of IDH mutation, the 2HG concentration is shown to be higher in patients with IDH-mutant glioma than in patients with IDH wild-type glioma (1). However, the diagnostic performance of 2HG MR spectroscopy for predicting IDH mutations in patients with glioblastoma (WHO grade 4), especially when compared with lower-grade gliomas, has not been validated (3). Measurements of 2HG in glioblastomas have shown relatively high rates of false-positive results (4–6). For example, in one study of 24 patients with glioma, it was found that four patients had false-positive results from 2HG measurements, with two having very high lactate concentrations (4). False-positive results based on 2HG concentrations may also be caused by necrosis (5). For example, 21% of IDH wild-type glioblastomas yielded false-positive results on 2HG assays, with a higher false-positive rate associated with tumor necrosis and an apparent diffusion coefficient value (6).

Necrosis is an inherent characteristic of glioblastoma, with necrotic areas having high lipid and lactate concentrations (3,7). Because 2HG concentrations are frequently measured using a single-voxel MR spectroscopy approach (1), it is difficult to avoid areas of necrosis when determining the volume of interest (VOI) in glioblastomas. Lipid concentrations are high in areas of necrosis, with some of
These lipids resonating between 2.0 and 2.9 ppm at MR spectroscopy (8). As 2HG concentrations are usually measured at 2.25 ppm, the resonance of these lipids can increase the signal recorded and lead to false-positive results from 2HG measurements. For example, a study of 17 patients with false-positive results from 2HG measurements found that 15 (88%) had lactate concentrations greater than or equal to 2.0 mmol/L and that 14 (82%) had lactate concentrations greater than or equal to 3.0 mmol/L (6). Because lipid and lactate are present in the necrotic portion and may be a primary cause of false-positive results from 2HG measurement, these and other findings (3–5) suggest that lipid and lactate are covariates that could be used to adjust the 2HG concentration. In addition, it is difficult to distinguish between WHO grades 3 and 4 glioma using pretreatment brain MRI, suggesting the need for consistent cutoff values for lower-grade gliomas and glioblastomas.

In this study, we therefore adjusted the 2HG concentration by using the lipid and lactate concentrations and calculated the 2HG-to-lipid and lactate (2HG/(lipid + lactate)) ratio and assessed whether this ratio could be validated as a potential diagnostic biomarker to predict the IDH mutation status in patients with lower-grade gliomas and glioblastomas. The purpose of this study was to compare the 2HG/(lipid + lactate) ratio with the 2HG concentration alone, as determined with 2HG MR spectroscopy, as predictors of the IDH mutation status in patients with glioma.

### Materials and Methods

#### Study Design

This study is reported in accordance with the Standards for Reporting of Diagnostic Accuracy Studies 2015 guidelines (9). This observational, retrospective study was approved by the institutional review board of the tertiary referral hospital, which waived the requirement for written informed consent because of the observational nature of this study. The study group consisted of 152 consecutive patients with histopathologically confirmed glioma (WHO grades 2–4) without prior treatment who underwent 2HG MR spectroscopy between July 2016 and February 2019 (2). Patients were included if they had been histopathologically diagnosed with glioma, had undergone 2HG MR spectroscopy without motion artifacts or hemorrhage, and had a known IDH mutation status. A total of 66 patients who did not undergo 2HG spectroscopy were excluded, leaving 86 patients with histologically confirmed glioma. Patients were excluded if major MR spectroscopy metabolites could not be analyzed for technical reasons (n = 5), the VOI consisted of pure necrosis, or their IDH mutation status was undetermined (n = 2). A total of 79 patients were enrolled in this study (Fig 1). These patients underwent 2HG MR spectroscopy a median of 8 days prior to surgery.

Twenty-three of the 79 participants (29%) were previously described (6) in a study dealing with factors associated with false-positive 2HG measurements in patients with IDH wild-type glioblastoma. Because the present study compared the abilities of the 2HG/(lipid + lactate) ratio and the 2HG concentration alone to predict the IDH mutation status in patients with glioma grades 2–4, the aim of this study differed from those of the previous studies.

### IDH Mutation Status

IDH1 (R132H) protein expression was assessed by using immunohistochemistry (10). Patients with negative immunohistochemistry results for IDH1 (R132H) were assessed with DNA pyrosequencing for R172K mutations in the IDH1 and IDH2 genes (10). The European Association for Neuro-Oncology guidelines state that a negative immunohistochemistry result for IDH1 (R132H) expression is sufficient for a classification of IDH wild-type glioblastoma in patients older than 55 years at diagnosis with typical primary glioblastoma (10). All immunohistochemistry and DNA pyrosequencing assays were performed by the pathology division of our hospital, without knowledge of the results of the 2HG measurement.

#### Protocol for 2HG MR Spectroscopy

Each 2HG MR spectroscopic examination was performed with a 3-T unit (Ingenia CX; Philips Medical Systems, Best, the Netherlands) using a 32-channel sensitivity-encoding head coil. The detailed protocols for 2HG MR spectroscopy have previously been described (6,11,12). In brief, T2-weighted images were obtained in the coronal and sagittal planes for accurate localization of the voxel. A single-voxel point-resolved spectroscopy (PRESS) sequence using a long echo time (TE) (TE, [32 msec] + TE, [65 msec] = total TE [97 msec]) was used, along with the following MR spectroscopic parameters: repetition time, 2000 msec; VOI, 2.0 × 2.0 × 2.0 cm; and 16 signal averages. The chosen VOI size of 2.0 × 2.0 × 2.0 cm was based on previous reports (4,5,11–14). The VOI was positioned by a neuroradiologist (C.H.S., with 6 years of clinical
experience in neuro-oncologic imaging) using axial, coronal, and sagittal T2-weighted images to include as much of the pure solid portion of the mass as possible while limiting the incorporation of normal parenchyma, the surgical cavity, the cerebrospinal fluid space, and areas of necrosis. Because necrosis is one of the key imaging findings of glioblastoma, inclusion of some necrosis within the VOI was inevitable; thus, we measured the necrosis volume within the VOI.

In each water-suppressed PRESS scan, an unsuppressed PRESS water signal was obtained with the same gradient scheme for multichannel combinations and eddy current compensation. In addition, an unsuppressed water signal was acquired using a stimulated-echo acquisition mode with the following parameters: repetition time, 18,000 msec; TE, 14 msec; and VOI, 2.0 × 2.0 × 2.0 cm. The total acquisition time for 2HG MR spectroscopy was 5 minutes 56 seconds.

Image Processing and Analysis
All images were processed and analyzed by a neuroradiologist (C.H.S.) blinded to the clinical information, results of other imaging tests, and reference standards, with supervision by another experienced neuroradiologist (H.S.K., with 21 years of clinical experience in neuro-oncologic imaging) using axial, coronal, and sagittal T2-weighted images to include as much of the pure solid portion of the mass as possible while limiting the incorporation of normal parenchyma, the surgical cavity, the cerebrospinal fluid space, and areas of necrosis. Because necrosis is one of the key imaging findings of glioblastoma, inclusion of some necrosis within the VOI was inevitable; thus, we measured the necrosis volume within the VOI.

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Statistical Analysis
The ability of several parameters to predict IDH mutations in patients with glioma without prior treatment was evaluated. These parameters included the 2HG concentration (3–5,11–13,19–21), ratio of 2HG to the total creatine concentration (14,17), ratio of 2HG to the glutamate plus glutamine concentration (22), ratio of total choline to total creatine concentration, and ratio of total choline to total N-acetylaspartate concentration. The diagnostic performance of each of these parameters was compared with that of the 2HG/(lipid + lactate) ratio.

The sensitivities, specificities, areas under the receiver operating characteristic curve (AUCs), and corresponding optimal cutoff values were calculated using the Youden index (23). The Youden index is defined as sensitivity + specificity − 1 (ranging from −1 to +1), with a value of +1 indicating the optimal value. Internal validation was assessed by the bootstrap approach, with 1000 bootstrap resamples used to adjust for the optimum. Optimum-corrected C statistics were obtained by subtracting the optimum from the apparent C statistics in the data set (24).

Improvements in predictive accuracy were evaluated by calculating the absolute integrated discrimination improvement (25). Patients were also subgrouped into those with lower-grade glioma (WHO grades 2 and 3) and glioblastoma (WHO grade 4) (2). All statistical analyses were performed using SAS (version 9.3, SAS Institute, Cary, NC) and SPSS (version 21.0; SPSS, Chicago, Ill), with P values less than .05 defined as statistically significant.

Results
Patient Characteristics
The demographic and clinical characteristics of the 79 patients are shown in Table 1. Of these 79 patients, 28 (35%) had IDH mutations and 51 (65%) had wild-type IDH. Thirty-two patients (41%) had lower-grade glioma, and 47 (59%) had glioblastoma. The mean necrosis volume within the VOI in glioblastoma was 0.8 cm³ ± 1.1 (standard deviation). Of the patients with lower-grade glioma, those with mutant IDH (mean age, 45 years ± 11) were younger than those with wild-type IDH (61 years ± 10, P < .01). Similarly, of the patients with glioblastoma, those with mutant IDH (44 years ± 10.9) were younger than those with wild-type IDH (59 years ±
Value of 2HG/(Lipid + Lactate) Ratio for Predicting IDH Mutation Status

9; \( P < .01 \). Among the 79 patients, 32 patients underwent gross total resection, 34 patients underwent partial resection, and 13 patients underwent biopsy using neuronavigation by a neurosurgeon.

In terms of data-quality measures, the mean value of the full width at half maximum of the spectra was 6.2 Hz \( \pm \) 2.8. The Cramer-Rao lower bounds for major metabolites were as follows: total creatine: mean, 3.4% \( \pm \) 2.4; total choline: mean, 1.8% \( \pm \) 1.2; N-acetylaspartate: mean, 5.5% \( \pm \) 4.4; 2HG: mean, 151.3% \( \pm \) 326.7; and lipid and lactate: mean, 12.1% \( \pm \) 19.7.

### Overall Diagnostic Performance

The 2HG concentration was higher in patients with mutant IDH gliomas (2.47 mmol/L \( \pm \) 1.68) than in patients with wild-type IDH gliomas (1.01 mmol/L \( \pm \) 1.07) \( (P < .01) \). The sensitivity and specificity of the 2HG concentration for the prediction of IDH-mutant glioma were 89% (25 of 28; 95% confidence interval [CI]: 72%, 98%) and 67% (34 of 51; 95% CI: 52%, 79%), respectively. The AUC was 0.80 (95% CI: 0.70, 0.88), and the optimal cutoff value for 2HG was 1.12 mmol/L.

Similarly, the 2HG/(lipid + lactate) ratio was higher in patients with mutant IDH gliomas (1.55 \( \pm \) 1.11) than in patients with wild-type IDH gliomas (0.30 \( \pm \) 0.45) \( (P < .01) \). The sensitivity and specificity of 2HG/(lipid + lactate) were 79% (22 of 28; 95% CI: 59%, 92%) and 92% (47 of 51; 95% CI: 81%, 98%), respectively. The AUC was 0.90 (95% CI: 0.81, 0.96). The diagnostic performances of the other parameters tested are shown in Table 2. The 2HG/(lipid + lactate) ratio had the highest AUC among these parameters. The optimal cutoff value for the 2HG/(lipid + lactate) ratio was 0.63.

These results were internally validated by 1000 bootstrap resamples from the data set. The optimum-corrected C statistics for 2HG and the 2HG/(lipid + lactate) ratio were 0.80 (95% CI: 0.71, 0.90) and 0.90 (95% CI: 0.83, 0.97), respectively. The integrated discrimination improvement showed that the 2HG/(lipid + lactate) ratio was better than the 2HG concentration for predicting IDH-mutant glioma \( (P < .01; \text{Table 3}) \).

### Subgroup Analyses in Patients with Glioblastomas and Lower-grade Gliomas

The sensitivity and specificity of the 2HG concentration for the prediction of IDH-mutant glioblastomas were 86% (six of seven; 95% CI: 42%, 100%) and 60% (24 of 40; 95% CI: 43%, 75%), respectively. The sensitivity and specificity of the 2HG/(lipid + lactate) ratio were 100% (seven of seven; 95% CI: 59%, 100%) and 60% (24 of 40; 95% CI: 43%, 75%), respectively. The AUC for predicting IDH-mutant glioblastoma was higher for the 2HG/(lipid + lactate) ratio (0.84 [95% CI: 0.70, 0.93]) than for 2HG (0.73 [95% CI: 0.58, 0.85]). The optimum-corrected C statistics for the 2HG/(lipid + lactate) ratio and 2HG were 0.83 (95% CI: 0.70, 0.97) and 0.71 (95% CI: 0.53, 0.90), respectively. The 2HG/(lipid + lactate) ratio was better for predicting IDH mutations than the 2HG concentration alone, according to the integrated discrimination improvement, with borderline significance \( (P = .052; \text{Table 3}) \). The results of a representative patient with IDH wild-type glioblastoma, which were false-positive when using the 2HG concentration assessment alone, were true-negative when using the 2HG/(lipid + lactate) ratio (Fig 2).

The AUCs for predicting IDH-mutant lower-grade gliomas were similar for 2HG (0.89 [95% CI: 0.73, 0.97]) and the 2HG/(lipid + lactate) ratio (0.88 [95% CI: 0.70, 0.96]). The two parameters showed comparable diagnostic performance in predicting IDH-mutant lower-grade gliomas, according to the integrated

### Table 1: Demographic and Clinical Characteristics of Patients

| Characteristic | Lower-grade Glioma \((n = 32)\) | Glioblastoma \((n = 47)\) |
|---------------|-------------------------------|---------------------------|
| Sex           |                               |                           |
| Men           | 14                            | 6                         |
| Women         | 7                             | 5                         |
| Age \(\pm SD\) (y) | 45 \(\pm 11\)           | 61 \(\pm 10\)             |
| Biopsy or surgical extent | 0.03                           | .66                       |
| Biopsy        | 2                             | 5                         |
| Partial resection | 13                            | 2                         |
| Gross total resection | 6                             | 4                         |
| Size (mm)     | 6.3                           | 6.6                       |
| Location      |                               |                           |
| Supratentorial| 21                            | 10                        |
| Infratentorial| 0                             | 1                         |

Note.—IDH = isocitrate dehydrogenase, SD = standard deviation.

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with lower-grade glioma showed comparable diagnostic performance of the two measurements. Therefore, the greater ability of the 2HG/(lipid + lactate) ratio in comparison with the 2HG concentration to predict IDH mutation status was due primarily to the former having a lower rate of false-positive measurements in glioblastoma. These findings suggest that the 2HG/(lipid + lactate) ratio may have the potential to be a diagnostic biomarker for predicting IDH mutation status.

Glioblastomas have high lactate and/or lipid concentrations due to necrosis (3,7). The peak at 1.3 ppm was higher in both IDH-mutant and wild-type IDH glioblastomas than in lower-grade gliomas (3,21). In this study, the diagnostic performance of the 2HG concentration was relatively low in glioblastomas (AUC = 0.73). Although previous studies have evaluated the predictive ability of various parameters, including the 2HG concentration (3–5,11–13,19–21), ratio of 2HG to the total creatine concentration (14,17), and ratio of 2HG to the glutamate plus glutamine concentration (22), these parameters have rarely been used to predict IDH mutation status in glioblastomas. The present study therefore validated the discrimination improvement (P = .72). Results in a representative patient with IDH-mutant glioma are shown in Figure 3.

**Discussion**

Although 2HG MR spectroscopy is used to predict IDH mutation status in patients with glioma, the presence of other metabolites, including lipid and lactate, may complicate these results. This study compared the abilities of 2HG/(lipid + lactate) ratio and 2HG concentration to predict IDH mutation status in patients with glioma. In the entire group of patients, the 2HG/(lipid + lactate) ratio (AUC = 0.90 [95% CI: 0.81, 0.96]) showed better diagnostic performance than the 2HG concentration (AUC = 0.80 [95% CI: 0.70, 0.88]) in predicting IDH mutation status (P < .01). According to the integrated discrimination improvement, the 2HG/(lipid + lactate) ratio showed improved diagnostic performance compared with the 2HG concentration for the prediction of IDH mutations. Similarly, subgroup analysis in patients with glioblastoma showed that the diagnostic performance of the 2HG/(lipid + lactate) ratio was better than that of the 2HG concentration. By contrast, subgroup analysis in patients with lower-grade glioma showed comparable diagnostic performance of the two measurements. Therefore, the greater ability of the 2HG/(lipid + lactate) ratio in comparison with the 2HG concentration to predict IDH mutation status was due primarily to the former having a lower rate of false-positive measurements in glioblastoma. These findings suggest that the 2HG/(lipid + lactate) ratio may have the potential to be a diagnostic biomarker for predicting IDH mutation status.

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2HG/(lipid + lactate) ratio as a prospective biomarker in untreated patients with both glioblastoma and lower-grade glioma.

This study showed that the diagnostic performance of 2HG/(lipid + lactate), including its sensitivity, specificity, and AUC, was significantly higher than that of the 2HG concentration in gliomas, especially in glioblastomas. In addition, the optimal cutoff value for the 2HG/(lipid + lactate) ratio was 0.63. The integrated discrimination improvement also demonstrated that, compared with the 2HG concentration, the 2HG/(lipid + lactate) ratio was better able to predict IDH mutations. Moreover, the 2HG/(lipid + lactate) ratio showed the highest AUC of all the parameters evaluated.

**Figure 2:** MR images in a 59-year-old man with IDH wild-type glioblastoma. (a) Axial T2-weighted and (b) FLAIR images show a large infiltrative mass in both frontal lobes and the right basal ganglia. The mass shows (c, d) heterogeneous enhancement and necrosis on contrast material–enhanced T1-weighted images and (e, f) diffusion restriction on diffusion-weighted images. (g) Sagittal, axial, and coronal T2-weighted images show the location of the volume of interest (2.0 × 2.0 × 2.0 cm), which contains areas of necrosis. (h) Spectra were obtained using point-resolved spectroscopy with a long echo time (97 msec), and spectral fitting was performed using LCModel software. The 2HG concentration was 1.32 mmol/L, which was a false-positive result. By contrast, the 2HG/(lipid + lactate) ratio was 0.11 and showed that the patient had true-negative results for IDH mutation. 2HG = 2-hydroxyglutarate, 2HG/(lipid + lactate) = 2HG-to–lipid and lactate, FLAIR = fluid-attenuated inversion recovery, FWHM = full width at half maximum, GABA = γ-aminobutyric acid, Glu = glutamate, Gly = glycine, IDH = isocitrate dehydrogenase, Lac = lactate, tCho = total choline, tCr = total creatine, tNAA = total N-acetylaspartate, ml = myo-inositol, SD = standard deviation. (Fig 2 continues)
Use of the 2HG/(lipid + lactate) ratio as a predictive biomarker may have several advantages over use of other parameters. First, the 2HG/(lipid + lactate) ratio is a simple and easily applicable parameter, which showed improved diagnostic performance in all grades of glioma, especially when patients with glioblastoma were included. The difficulty of diagnosing grades 3 and 4 gliomas using pretreatment brain MRI suggests the need for a consistent parameter with a corresponding cutoff value for lower-grade gliomas and glioblastomas. Second, the 2HG/(lipid + lactate) ratio can account for differences in lipid and lactate concentrations, which are indicators of necrosis. Thus, the 2HG/(lipid + lactate) ratio may be applicable when the VOI used for 2HG MR spectroscopy contains necrotic areas. Third, the 2HG/(lipid + lactate) ratio can account for differences in lipid and lactate concentrations, which are indicators of necrosis. Thus, the 2HG/(lipid + lactate) ratio may be applicable when the VOI used for 2HG MR spectroscopy contains necrotic areas. Fourth, 2HG, lipid, and lactate concentrations can be measured simultaneously, with no need to combine or co-register images with those of another imaging modality. Taken together, these findings suggest that the 2HG/(lipid + lactate) ratio can be used clinically to predict IDH mutation status, especially in patients with glioblastoma prior to treatment.

The use of 2HG MR spectroscopy has mostly been for determination of IDH mutations in lower-grade gliomas. Of the 14 studies included in a previous meta-analysis, 13 included patients with lower-grade glioma and nine evaluated the 2HG concentration as a predictive parameter (1). Although multiple parameters were combined, 2HG MR spectroscopy demonstrated excellent diagnostic performance in the prediction of IDH mutation status, with a pooled sensitivity of 95% (95% CI: 85%, 98%) and a pooled specificity of 91% (95% CI: 83%, 96%) (1). The 2HG concentration is therefore a well-validated and simple parameter for predicting IDH mutation status in lower-grade gliomas. The present study showed that the 2HG/(lipid + lactate) ratio and 2HG concentration had comparable diagnostic performance in lower-grade gliomas, indicating the usefulness of the 2HG/(lipid + lactate) ratio, even in lower-grade gliomas.

This study had several limitations. First, in our protocol, we could not separate lipid and lactate peaks, which included a TE of 97 msec. Further studies using emerging 2HG MR spectroscopic protocols or postprocessing methods that may enable separation of lipid and lactate peaks are necessary. Second, the number of patients (n = 79) was relatively small. Third, the study only included previously untreated patients. To determine the clinical applicability of this parameter, it should be evaluated in patients with glioma after treatment. Studies in large cohorts, including patients who have and have not been treated previously, are therefore warranted.
Fourth, there might be potential bias, given that the number of patients with glioblastoma could affect the accuracy of the 2HG/(lipid + lactate) ratio. However, we included consecutive patients with histopathologically confirmed glioma (WHO grades 2–4). Last, this study might be vulnerable to selection bias. In our study, 66 of 152 patients (43.4%) did not undergo 2HG MR spectroscopy, and we had seven of 47 (14.9%) patients with glioblastoma with mutant IDH. This is probably due to the fact that 2HG MR spectroscopy is performed by the order of referring physicians. Further large studies of consecutive patients who have undergone 2HG MR spectroscopy are needed.
In conclusion, this study showed that, at 2HG MR spectroscopy, the 2HG/(lipid + lactate) ratio was more accurate in predicting IDH mutation status in patients with glioma than the 2HG concentration alone.

**Author contributions:** Guarantors of integrity of entire study, H.S.K., D.C.W.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, C.H.S., H.S.K., J.E.P., D.C.W., H.B.L.; clinical studies, C.H.S., H.S.K., J.E.P., S.C.J., C.G.C., D.C.W., H.B.L., S.J.K.; statistical analysis, C.H.S., H.S.K., J.E.P., D.C.W., H.B.L.; and manuscript editing, C.H.S., H.S.K., J.E.P., C.G.C., H.B.L.

**Disclosures of Conflicts of Interest:** C.H.S. disclosed no relevant relationships. H.S.K. disclosed no relevant relationships. J.E.P. disclosed no relevant relationships. S.C.J. disclosed no relevant relationships. C.G.C. disclosed no relevant relationships. D.C.W. disclosed no relevant relationships. H.B.L. disclosed no relevant relationships. S.J.K. disclosed no relevant relationships.

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