Myoinositol to total choline ratio in *IDH* wild-type gliomas as a prognostic factor on preoperative magnetic resonance spectroscopy

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Abstract

Background: Isocitrate dehydrogenase (IDH) wild-type gliomas tend to be pathologically defined as glioblastomas. We previously reported that, unlike *IDH*-mutant gliomas, *IDH* wild-type gliomas showed significantly lower ratios of myoinositol to total choline (i.e., the Ins/Cho ratio) on magnetic resonance (MR) spectroscopy. Given that *IDH*-mutant gliomas also have much better prognoses than *IDH* wild-type gliomas, we hypothesized that this lower Ins/Cho ratio is associated with malignancy in adults with supratentorial gliomas. Therefore, we calculated the Ins/Cho ratios of patients with supratentorial *IDH* wild-type gliomas and investigated their progression free survival (PFS) and overall survival (OS) to determine its utility as a prognostic marker.

Methods: We classified *IDH* wild-type gliomas (n = 30) into two groups based on the Ins/Cho ratios, and compared patient backgrounds, pathological findings, PFS, OS, and copy number aberrations.

Results: Compared with the group with high Ins/Cho ratios, the group with low Ins/Cho ratios had shorter PFS (P = 0.020) and OS (P = 0.037) durations. Multivariate analysis demonstrated that the Ins/Cho ratio correlated significantly with PFS (hazard ratio 0.34, P = 0.027).

Conclusion: We conclude that the preoperative Ins/Cho ratio can be used as a novel prognostic factor for *IDH* wild-type gliomas.

Background

Gliomas, which are diagnosed based on pathological and genetic findings, are among the most common brain tumors [1-4]. Extensive, surgical resection is the preferred initial therapeutic strategy to improve prognosis [5, 6]; however, such an approach also increases the risk of brain dysfunction because either the border between the tumor and normal brain tissue is unclear or because tumors infiltrate invasively into adjacent healthy brain tissue. Therefore, the ability to predict tumor malignancy preoperatively could help surgeons to plan optimal surgical strategies.

Magnetic resonance (MR) spectroscopy enables us to quantify tumor metabolites noninvasively by analyzing their spectra, and thus, it is widely used for preoperative diagnosis of brain tumors [7]. We previously examined tumor metabolites in adult supratentorial gliomas and reported that the myoinositol to total choline (Ins/Cho) ratios in isocitrate dehydrogenase (IDH) wild-type gliomas were significantly lower than in *IDH*-mutant gliomas [8]. Given that total choline is thought to represent cell density [9], we presumed that lower ratios indicate myoinositol consumption in a glioma cell. Myoinositols are sugar alcohol in phospholipids of the intracellular membrane [10], and they are produced through food digestion or by hydrolysis or dephosphorylation of the intracellular membrane [10-12]. In astrocytes, myoinositols contribute to adjusting osmotic pressure in response to changes in intracranial pressure [13].

Most supratentorial gliomas harboring wild-type *IDH* are pathologically classified as glioblastomas and tend to have dismal prognoses [1-3]. Molecular factors that contribute to this poor prognosis have been identified based on genetic subtype, including *TERT* promoter mutations, copy number aberrations (e.g., +7, 10q and 9p21), and epigenetic changes (e.g., histone H3) [14-18]. However, such information is only available after surgical resection, and we ideally need a method for predicting outcomes in patients suspected of having glioblastomas before surgery. Based on the observation that supratentorial gliomas with *IDH* mutations have better outcomes than those with wild-type *IDH* [1, 2, 3], we hypothesized that low Ins/Cho ratios in *IDH* wild-type gliomas can be used as a prognostic marker for this genetic subtype of glioma.

In the present study, we calculated Ins/Cho ratios by MR spectroscopy for adults with supratentorial *IDH* wild-type gliomas to investigate the ratio's association with prognosis, its correlation with previously reported prognostic factors, and whether it could be used as a preoperative prognostic factor.

Materials And Methods

Study design

We retrospectively investigated 30 cases of newly diagnosed *IDH* wild-type glioma in patients analyzed by MR spectroscopy between 2013 and 2018 at Fujita Health University hospital. Patients who had already received treatment before MR spectroscopy or who were younger than 20 years old were excluded.

The primary outcomes were the progression free survival (PFS) and the overall survival (OS). PFS was calculated from the date of first operation to the date of confirmed recurrence. OS was calculated from the date of first resection to the date of death. Patient information was last updated in April 2019 for follow-up purposes. All resected tissues were assessed by neuropathologists according to the WHO classification [1].

MR spectroscopy

A single-voxel ¹H-MR spectroscopy with point-resolved spectroscopy sequence was performed with a 3 Tesla (T) scanner (Ingenia 3T; Philips Healthcare, Best, The Netherlands) using a dS Head coil and Vantage Titan 3T (Canon Medical Systems Corporation, Otawara, Japan) using a 16 or a 32-channel coil. In each patient, ¹H-MR spectroscopy was performed using the following parameters: repetition time (TR)/echo time (TE), 2000/144 and 35 ms; number of excitation (NEX), 128; bandwidth, 1.61 HZ/point; voxel of interest (VOI) size for metabolic measurements, 15 × 15 × 15 mm. T2 weighted images in 3 directions for setting the VOI were determined for each patient by means of the following parameters: repetition time (TR)/echo time (TE), 4250/82 ms; acquisition matrix size, 416 × 344; reconstruction matrix, 640 × 640; field of view (FOV), 230 × 230 mm; slice thickness, 4.0 mm; slice gap, 0.8 mm; number of excitation (NEX), 1; reduction factor 1.9. The VOI was selected by a board certified neuroradiologist (K.M) with an experience of 10–15 years' to include the VOIs inside the lesion based on T2 weighted

images in 3 directions. When the tumor contains necrotic and cystic components, these components were included within VOI to accurately evaluate tumor characteristics. In ^1H -MR spectroscopy, myoinositol and total choline were measured with short TE (TE = 35 ms) and long TE (TE = 144 ms) respectively [19, 20, 21] because of their relaxation time in T2, and mean concentration of MR spectroscopy values were analyzed by automatic quantification program (LCModel; Stephen Provencher, Oakville, Ontario, Canada) [22].

Evaluation of *IDH* mutation status

We evaluated *IDH* mutations using the Sanger method, with codon 132 in *IDH1* and codon 172 in *IDH2* analyzed by polymerase chain reaction (PCR). Briefly, DNA was extracted from resected frozen tissue and formalin-fixed and paraffin-embedded tissue (FFPE), using DNeasy blood and tissue kits (QIAGEN, Hulsterweg, Netherland) and an REPLI-g FFPE Kit (QIAGEN). The reaction mixtures for PCR comprised DNA, primers, 10PCR buffer, 10mM dNTP mix (Thermo Fisher Scientific, Waltham, MA, **USA**), 50 mM MGCL $\times$, and PLATINUM TagDNA polymerase (Thermo Fisher Scientific). After we confirmed the DNA bands of the PCR products in electrophoresis, we added BigDye Sequencing Buffer (Thermo Fisher Scientific), Ready Reaction Mix (Thermo Fisher Scientific), and the same primer to the PCR products, and repeated the PCR. Sequencing was performed with a BigDye Terminator version 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and results were analyzed on an ABI 3100 (Applied Biosystems, Waltham, MA, **USA**).

Next generation sequencing analysis and comparison of Copy number aberration

We analyzed samples that met our inclusion criteria and classified them by Ins/Cho ratio into high and low and a low ratio groups. We prepared diluted genomic DNA for subsequent experiments. After whole genome amplification using a SurePlex DNA Amplification System (Illumina, San Diego, CA, **USA**), library preparation was performed with a Nextera XT DNA Library Preparation Kit (Illumina). Sequencing analysis was conducted with a VeriSeq PGS Kit-MiSeq (Illumina), and results were analyzed with BlueFuse Multi Software (Illumina). In all cases, chromosomes were divided into 2500 windows of approximately 1 Mb in size.

Statistical analysis

We used Fisher's exact test and the MannWhitney *U* test to compare age at onset, sex, laterality, MIB-1 index, and pathology between the two groups. PFS and OS were analyzed using the KaplanMeier method and compared with the log-rank test. Cox proportional hazards models were used to determine the relationship between the Ins/Cho ratio and prognosis. Given that most patients were treated with surgery, temozolomide and radiotherapy, and bevacizumab, we selected the following as explanatory factors in the multivariate analysis: gross total resection; subtotal resection, which was defined as >90% resection; temozolomide and radiotherapy; bevacizumab; and the Ins/Cho ratio. All statistical analyses were conducted using EZR [23].

Results

Relation of patient and pathology characteristics with the Ins/Cho ratio

The mean and median values of the Ins/Cho ratios for the 30 patients included in this cohort were 0.75 and 0.67, respectively. We opted for 0.7 as the cutoff value and classified those patients into high (≥ 0.7) and low (< 0.7) Ins/Cho ratio groups (n = 13 and 17, respectively). Tables 1 and 2 show the patient characteristics, pathologies, Ins/Cho ratios, and postoperative therapies. Case comprised 25 glioblastoma, two gliosarcomas, three astrocytomas. Two of three astrocytomas gained chromosome 7 and/or loss of chromosome 10q, but the other was not associated with them. There were no statistically significant differences in sex, age at onset, laterality, MIB-1 index, or grade $\times$ WHO classification between the two groups. Overall, eight patients underwent reoperation, six patients underwent additional chemotherapy, and seven patients had no treatment after recurrence.

Table 1. Characteristics and treatments of patients

Case	Age, sex	Pathology	Recurrence	PFS	Follow-up terms	Outcome	Ins/Cho
First operation / Postoperative therapies / therapies after recurrence							
1	42F	GBM, Gr II	Yes	9	22	Dead	0.573
GTR / TMZ + RT / TMZ + RT + BEV+ other chemotherapies + operation							
2	43M	GBM, Gr II	Yes	5	12	Dead	0.475
PR / TMZ + RT / BEV + another chemotherapy + operation							
3	43F	GBM, Gr II	Yes	1	31	Dead	0.507
GTR / No treatment / TMZ + RT + BEV + other chemotherapies + operations							
4	44M	GBM, Gr II	Yes	10	14	Dead	0.167
GTR / TMZ + RT + BEV / other chemotherapy							
5	46F	GBM, Gr II	Yes	3	12	Dead	0.450
GTR / TMZ + RT / TMZ + RT + BEV + other chemotherapy							
6	62M	GBM, Gr II	Yes	14	49	Dead	0.516
Biopsy / TMZ + RT / TMZ + RT + BEV + operation							
7	62M	GS, Gr II	Yes	26	58	Alive	1.149
STR / TMZ + RT / TMZ + RT + operation							
8	63F	GBM, Gr II	Yes	17	36	Alive	1.149
GTR / TMZ + RT / TMZ + RT + BEV							
9	67M	GBM, Gr II	Yes	2	2	Alive	0.044
PR / TMZ + RT / BEV							
10	67M	GBM, Gr II	Yes	9	10	Alive	0.816
PR / TMZ + RT + BEV / TMZ							
11	68F	GBM, Gr II	Yes	8	8	Dead	0.227
GTR / TMZ + RT / No treatment							
12	68M	GBM, Gr II	NO	6	10	Alive	1.182
STR / TMZ + RT / No treatment							
13	69F	GBM, Gr II	Yes	1	12	Dead	2.054
GTR / No treatment / TMZ + RT + BEV							

14	69F	GBM, Gr II	Yes	12	20	Dead	0.655
GTR / TMZ + RT / BEV							
15	70M	GBM, Gr II	NO	3	11	Dead	0.854
GTR / TMZ + RT / No treatment							
16	71M	DA, Gr II	Yes	9	39	Dead	1.702
Biopsy / TMZ + RT / operation							
17	72M	GBM, Gr II	Yes	1	22	Dead	0.451
GTR / TMZ + RT / TMZ + RT + BEV							
18	72F	DA, Gr II	Yes	4	7	Alive	0.952
PR / No treatment / No treatment							
19	73F	GBM, Gr II	Yes	1	23	Dead	0.405
STR / No treatment / TMZ + RT + BEV + operation							
20	74M	GBM, Gr II	Yes	2	20	Dead	0.758
GTR / TMZ + RT / TMZ + BEV							
21	74M	GBM, Gr II	Yes	1	20	Dead	0.240
GTR / TMZ + RT / TMZ + BEV + another chemotherapy + Operation							
22	74M	GBM, Gr II	Yes	10	19	Alive	0.893
STR / TMZ + RT + BEV / TMZ + RT + BEV							
23	75F	GBM, Gr II	NO	6	6	Alive	0.405
GTR / TMZ + RT							
24	76F	AA, Gr II	Yes	1	24	Dead	0.635
Biopsy / TMZ + RT / TMZ + BEV							
25	77M	GS, Gr II	Yes	13	14	Dead	0.695
GTR / TMZ + BEV / No treatment							
26	80F	GBM, Gr II	NO	39	39	Alive	1.176
PR / TMZ + RT							
27	81M	GBM, Gr II	Yes	2	3	Dead	0.692
Biopsy / TMZ + BEV / No treatment							
28	81M	GBM, Gr II	Yes	4	4	Alive	1.397

GTR / No treatment							
29	85M	GBM, Gr Ⅲ	Yes	2	5	Alive	0.279
Biopsy / TMZ + BEV +RT / TMZ							
30	86F	GBM, Gr Ⅲ	Yes	21	24	Dead	1.111
STR / TMZ / BEV							

Data show the patient and treatment details after the first operation and after recurrence for supratentorial *IDH* wild-type glioma (n=30). Pathology was diagnosed according to the WHO classification 2016. Abbreviations: AA, anaplastic astrocytoma; BEV, bevacizumab; DA, diffuse astrocytoma; F, female; GBM, glioblastoma; Gr, grade; GS, gliosarcoma; GTR, **Gross total resection**; Ins/Cho, ratio of myoinositol to total choline; IDH, isocitrate dehydrogenase; M, male; PFS, progression free survival; PR, Partial resection; RT, radiotherapy; STR, Subtotal **resection**; TMZ, temozolomide.

Table 2. Comparison of patient characteristics by Ins/Cho ratio threshold

	Ins/Cho ratio $\geq$ 0.7 (n = 13)	Ins/Cho ratio<0.7 (n = 17)	P value
Male, n	8	9	0.72
Age at onset, median (years)	71	69	0.38
Left side, n	8	7	0.46
MIB-1 index, median	38	46	0.41
Grade Ⅲ, n	11	16	0.57

Abbreviations: Grade Ⅲ, grade Ⅲ in WHO Classification; Ins/Cho, ratio of myoinositol to total choline; n, number of patients.

PFS and OS analysis

We compared prognosis by the Ins/Cho ratio. During follow-up, tumor recurrence occurred in 94% of patients with low Ins/Cho ratio (i.e., 16/17) and in 77% with high Ins/Cho ratio (i.e., 10/13). Moreover, 82% (14/17) of patients with low ratios and 38% (5/13) with high ratios died. Figures 1a and 1b show that the PFS and OS in those with high ratios were significantly shorter than in those with low ratios, indicating higher recurrence and mortality rates in the patients with low Ins/Cho ratios (P = 0.020 and

0.037, respectively). In the multivariate analysis, Cox proportional hazards models revealed that the Ins/Cho ratio was significantly associated with PFS (hazard ratio 0.34, P = 0.027), which suggested that the Ins/Cho ratio was useful outcome predictor, especially for PFS. These data are summarized in Tables 3a and 3b.

Table 3a. Multivariate analysis of PFS

Explanatory factor	Hazard ratio (95% CI)	P value
GTR or STR	0.84 (0.36-2.0)	0.68
Use of BEV	0.76 (0.26-2.2)	0.62
Both use of TMZ and RT	0.52 (0.20-1.4)	0.18
Ins/Cho ratio	0.34 (0.13-0.88)	0.027

Table 3b. Multivariate analysis of OS

Explanatory factor	Hazard ratio (95% CI)	P value
GTR or STR	1.6 (0.44-5.6)	0.48
Use of BEV	0.91 (0.28-3.0)	0.88
Both use of TMZ and RT	0.70 (0.22-2.2)	0.55
Ins/Cho ratio	0.33 (0.10-1.1)	0.067

Tables 3a and 3b show the results of multivariate analysis: we chose GTR or STR, BEV, TMZ and RT, and the Ins/Cho ratio as explanatory factors and analyzed their relevance to PFS and OS. Abbreviations: BEV, bevacizumab; GTR, Gross total resection; Ins/Cho, ratio of myoinositol to total choline; OS, overall survival; PFS, progression free survival; RT, radiotherapy; STR, subtotal resection; TMZ, temozolomide.

Analysis of copy number aberration by next generation sequencing

We included 20 cases in the next generation sequencing, analyzing nine samples in the group with high Ins/Cho ratios (≥ 0.7) and 11 samples in the group with low Ins/Cho ratios (< 0.7). The overall noise value was < 0.3 . Analysis focused on regions where specific genes exist that are associated with poor prognosis in glioblastoma, including 7p11.2 (*EGFR*), 9p21.3 (*p16*), 10q23.3 (*PTEN*) [15, 16]. Gain of 7p11.2 was detected in seven cases with high ratios and in six cases with low ratios, loss of 9p21.3 was detected in four cases with high ratios and in three cases with low ratios, and loss of 10q23.3 was detected in four

cases with high ratios and in three cases with low ratios (Table 4). There were no significant differences between the groups in any other window.

Table 4. Comparison of copy number aberrations by Ins/Cho ratio

	Ins/Cho ratio ≥ 0.7	Ins/Cho ratio < 0.7	P value
	(n = 9)	(n = 11)	
+7p11.2	7 (78%)	6 (55%)	0.37
−9p21.3	4 (44%)	3 (27%)	0.64
−10q23.3	4 (44%)	3 (27%)	0.64

Copy number aberrations identified by next generation sequencing are compared by the Ins/Cho ratio. We defined an effective change of gain or loss when the average value of a window was >2.5 or <1.5 .

Abbreviations: Ins/Cho, ratio of myoinositol to total choline.

Discussion

In this study, we demonstrated that a high Ins/Cho ratio on MR spectroscopy was associated with a better prognosis than a low Ins/Cho ratio according to KaplanMeier curves. The Ins/Cho ratio was significantly associated with PFS by KaplanMeier and multivariate analysis. Of the three cases in which the Ins/Cho ratios exceeded 1.0 and that started the Stupp regimen soon after maximum safe resection, the PFS durations were 26, 17, and ≥ 39 months (Cases 7, 8, and 26, respectively). Given that total choline in MR spectroscopy reveals cell density [9], low myoinositol levels are considered associated with poor prognosis. Indeed, a recent cohort study of recurrent glioblastoma treated with bevacizumab reported that a high myoinositol value on MR spectroscopy was associated with a good prognosis [24]. Given all these results, we considered that myoinositol affects as a second messenger and has antitumor effects. Myoinositols are located in glial cells, especially in astrocytes [25], and regulate intracranial osmotic pressure [13]. Phosphatidylinositol 3-phosphate, which contains myoinositols in its structure, is produced by intracellular membrane metabolism [10] and activates the PI3K-Akt pathway as a second messenger [26]. Activation of this pathway may cause a poorer prognosis in patients with a low Ins/Cho ratio by prompting cell proliferation and myoinositol consumption. As shown in a previous study, oral administration of myoinositols can inhibit malignant transformation of tumor cells in patients suffering non-small-cell lung cancer, in which the PI3K-Akt pathway is important [27]. Other researchers also reported that myoinositols have antitumor effects that result from their phosphorylated metabolites, such as **inositol 1,3,4,5,6**-pentaphosphate and **inositol** hexaphosphate, which can induce tumor cell apoptosis [28, 29]. These previous studies suggest that malignancy in *IDH* wild-type gliomas with low Ins/Cho ratios is associated with a simple reduction in antitumor effects.

However, multivariate analysis only showed that the Ins/Cho ratio was significantly associated with PFS and not OS (Figure 1, Table 3). We assumed that the therapeutic strategies used after recurrence affected these results in the multivariate analyses. Indeed, some patients chose both surgical resection and additional chemotherapy (13%), whereas other patients opted for no additional therapy after recurrence (23%), potentially affecting outcomes. Notably, all patients younger than 50 years at diagnosis were in the group with a low Ins/Cho ratio, and 60% of these chose surgical resection and chemotherapy (Table 1). Two of these patients (cases 1 and 3) survived for approximately 2 years despite having low Ins/Cho ratios (0.57 and 0.51, respectively). By contrast, 20% of patients older than 60 years at diagnosis underwent surgical resection, and only 4% of these chose additional chemotherapy. Patients who opted for no therapy after recurrence had short survival time (e.g., cases 15 and 27) despite having relatively high Ins/Cho ratios (0.85 and 0.69, respectively).

Copy number analysis by next generation sequencing revealed no significant correlations between the Ins/Cho ratio and chromosomal areas, despite the association of specific copy number aberrations with glioblastoma (Table 4). Previous studies have shown that such copy number aberrations are an early stage of tumorigenesis in glioblastoma [15, 30, 31], and our results suggest that low myoinositol levels are less likely to be caused by decreased myoinositol synthesis due to chromosomal change and gene expression. Rather, the low myoinositol levels can be considered to result from consumption due to tumor growth.

This study is limited by its retrospective cohort design and lack of control of therapeutic strategies (e.g., resection extent by tumor location, postoperative bevacizumab use, and radiological dosages) or postoperative complications (e.g., delayed wound healing or high-fever affected the start of adjuvant therapy). These factors could have significantly affected the clinical courses and outcomes. We must now investigate more cases to clarify the putative correlations between the Ins/Cho ratio and patient prognosis.

Conclusions

The noninvasive Ins/Cho ratio can serve as a novel prognostic marker for adults with supratentorial *IDH* wild-type gliomas, providing useful preoperative information in patients with suspected glioblastomas. However, we can only speculate on why myoinositols are associated with patient outcomes, and questions around this will be targeted in future research.

Abbreviations

FFPE: formalin-fixed and paraffin-embedded tissue

IDH: isocitrate dehydrogenase

Ins/Cho ratio: ratio of myoinositol to total choline

MR: Magnetic resonance spectroscopy

OS: overall survival

PCR: polymerase chain reaction

PFS: progression free survival

VOI: voxel of interest

Declarations

Ethical approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local ethical review board of Fujita Health University (HG19-017).

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

MK is the author and who conducted this study. TK, HS, HK conducted the copy number analysis and drafted the manuscript. KM and YO analyzed metabolites of glioma in this study and drafted the manuscript. MA and SY diagnosed tissue pathologically according to the WHO classification. SN, SO, JI, MH, and YH revised and edited the manuscript.

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Figures

Figure 1a

Figure 1b

Figure 1

Comparison of PFS (a) and OS (b) between the group with a high Ins/Cho ratio ($n = 13$) and that with a low ratio ($n = 17$). Analysis was by the Kaplan–Meier method, using the log-rank test. Abbreviations: Ins/Cho, ratio of myoinositol to total Choline; OS, overall survival; PFS, progression free survival.