Original paper

Folia Neuropathologica

The diagnostic challenge of lack of choline level elevation on ^{1}H-MR spectroscopy in grade II-III gliomas

Barbara Bobek-Billewicz[1], Sylwia Heinze[2], Krzysztof Majchrzak[3], Patrycja Mazgaj[1], Anna Hebda[1]

[1]Department of Radiology, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Gliwice Branch, Poland,
[2]Department of Radiology, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Cracow Branch, Poland,
[3]Department and Clinical Ward of Neurosurgery, Medical University of Silesia, Sosnowiec, Poland

Folia Neuropathol 2024; 62 (1): 13-20 *DOI: https://doi.org/10.5114/fn.2024.136469*

Abstract

The accurate diagnosis of brain tumour is very important in modern neuro-oncology medicine. Magnetic resonance spectroscopy (MRS) is supposed to be a promising tool for detecting cancerous lesions. However, the interpretation of MRS data is complicated by the fact that not all cancerous lesions exhibit elevated choline (Cho) levels. The main goal of our study was to investigate the lack of Cho_{lesion}/Cho_{ref} elevation in the population of grade II-III gliomas.
89 cases of gliomas grade II and III were used for the retrospective analysis – glioma (astrocytoma or oligodendroglioma) grade II (74 out of 89 cases [83%]) and III (15 out of 89 cases [17%]) underwent conventional MRI extended by MRS before treatment. Histopathological diagnosis was obtained either by biopsy or surgical resection. Gliomas were classified to the group of no-choline elevation when the ratio of choline measured within the tumour (Cho_{lesion}) to choline from NABT (Cho_{ref}) were equal to or lower than 1. Significant differences were observed between ratios of Cho_{lesion}/Cr_{lesion} calculated for no-choline elevation and glial tumour groups as well as in the NAA_{lesion}/Cr_{lesion} ratio between the no-choline elevation group and glial tumour group. With consistent data concerning choline level elevation and slightly lower NAA value, the $Cho_{lesion}/NAA_{lesion}$ ratio is significantly higher in the WHO II glial tumour group compared to the no-choline elevation cases ($p < 0.000$).
In the current study the results demonstrated possibility of lack of choline elevation in patients with grade II-III gliomas, so it is important to remember that the lack of elevated choline levels does not exclude neoplastic lesion.

Key words: *MRS, MRI, brain tumour, glioma grade, choline.*

Introduction

Proton magnetic resonance spectroscopy (^{1}H-MRS) is an analytical method used for the identification and quantification of metabolites. It allows a non-invasive, *in vivo* assessment of metabolites in tissue, complementing the anatomical information obtained with conventional magnetic resonance imaging (MRI) [2,18,20]. Because glial tumours have some specific metabolic characteristics that change according to the tumour grade, MRS can increase the sensitivity of standard diagnostic imaging [5,14,17].

Gliomas are the most common primary neoplasms of the central nervous system (CNS), with a different genotype, phenotype and grade [7]. According to 2021 CNS5 World Health Organisation (WHO) classification, Gliomas, Glioneuronal Tumours and Neuronal Tumours are divided into 6 different groups [11]. Histopatho logical and molecular diagnosis in brain tumours is achieved by a neurosurgical procedure or stereotac-

Communicating author:
Sylwia Heinze, PhD, Department of Radiology, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Cracow Branch, Poland, e-mail: sylwia.heinze@onkologia.krakow.pl

Received: 27.06.2023, **Accepted:** 23.11.2023, **Online publication:** 25.03.2024

tic biopsy, which could be associated with side effects (intracranial bleeding, neurological deficits), samples might not be taken from the most malignant part of the tumour. Imaging makes it possible to assess the entire tumour. MRI in conjunction with spectroscopy seems to be an important complement to invasive procedures especially in cases of doubt as to whether the lesion is a neoplastic tumour. Magnetic resonance spectroscopy is often used for differential diagnosis and glioma grading of intra-axial lesions. Typical ^{1}H-MRS findings of cerebral gliomas include elevation of choline (Cho), reduction of N-acetyl-aspartate (NAA), varying levels of creatine (Cr), lactate (Lac) and lipids. Choline is a precursor of acetylcholine (ACH) and a cell membrane. It resonates at 3.2 ppm chemical shift. Choline is a metabolic marker of cellular proliferation and cell membrane integrity. An elevated choline level is adequate to increase cell membrane synthesis and degradation. It was found that an increase in the choline level is present in brain tumours due to increased membrane turnover [3].

Statistically significantly higher values of Cho/Cr and Cho/NAA are observed in high-grade than in low-grade gliomas. Although increased choline is related to the tumour grade, few grade IV gliomas have lower choline levels than grade II or grade III gliomas. This may be caused by the presence of necrosis in high-grade tumours because necrosis is correlated with a notable lipid peak and decreased levels of the other metabolites [10].

The precise diagnosis of brain tumours is a critical challenge within contemporary neuro-oncology medicine. Magnetic resonance spectroscopy has emerged as a promising tool for the identification of cancerous lesions. Nevertheless, the interpretation of MRS data is complicated because not all cancerous lesions exhibit elevated choline levels. The uncertainty and diagnostic confusion associated with this issue have significant implications, potentially resulting in serious consequences for patients. The presence or absence of choline elevation in gliomas offers crucial insights into the tumour grade and aggressiveness, given that choline serves as a marker for malignant tissue. However, it is important to note that gliomas with low choline levels may not definitively exclude the presence of a brain tumour. In cases where elevated choline levels are not detected in MR spectra, the initial inclination is to consider a non-neoplastic lesion diagnosis, which, if incorrect, can lead to misdiagnoses. Therefore, the primary objective of our study is to investigate the absence of Cho_{lesion}/Cho_{ref} elevation in the population of grade II-III gliomas.

This has led to uncertainty and diagnostic confusion, with potentially serious consequences for patients. The presence or absence of choline elevation in gliomas can provide important information about tumour grade and aggressiveness since choline is a marker for malignant tissue. However, it is possible for gliomas with low choline levels not to exclude brain tumour. The main objective of our study was to investigate the lack of Cho_{les}/Cho_{ref} elevation in the population of grade II-III gliomas.

Material and methods

Over a span of ten years, 417 cases involving grade II and III gliomas were identified. Among these patients, we selected those who exhibited supratentorial tumour localization without ependymomas, with tumour dimensions suitable for voxel insertion not smaller than 1 × 1 × 1 cm, and positioned in a way that allowed for the acquisition of high-quality diagnostic MR spectra (both from the lesion and reference regions) with an adequately high signal-to-noise ratio (SNR) of no less than 2. Within the specified timeframe of 1.5 years, 89 patients met these criteria, with a median age of 41 years (with a quartile range of 33-47 years).

Patients with glioma (astrocytoma or oligodendroglioma) grade II (74 out of 89 cases [83%]) and III (15 out of 89 cases [17%]) underwent conventional magnetic resonance imaging extended by MRS before treatment. The patients included in the study had histologically confirmed diagnosis of grade II and III glioma (Table I). All cases with poor quality of the obtained ^{1}H-MRS spectra or a lack of reliable histopathological verification were excluded from the cohort.

The histopathological diagnosis was obtained either by biopsy or surgical resection (Table I). All tumours

Table I. Histological diagnosis of grade II-III gliomas

Histopathology of neoplasms	Grade	Number of patients	Percentage of the group [%]
Gemistocytic astrocytoma	II	2	2.2
Diffuse astrocytoma	II	42	47.2
Fibrillary astrocytoma	II	11	12.3
Mixed glioma (oligo-astrocytoma)	II	6	6.7
Oligodendroglioma	II	13	14.6
Anaplastic oligodendroglioma	III	5	5.6
Anaplastic astrocytoma	III	10	11.2

enrolled into the study were graded according to the currently valid WHO criteria.

Methods

The cuboid-shaped ^{1}H-MRS voxel was placed based on 3D images within the solid part of the tumour, with avoidance of its cystic/necrotic areas, bleeding, skull bones and ventricles. The reference voxel was placed contralaterally in normal-appearing brain tissue. Surgical intervention included complete resection of the brain tumour or partial resection if the tumour was located near sensitive areas of the brain to alleviate negative symptoms and facilitate recovery.

Data acquisition

Conventional MRI

All patients underwent MRI on a 3T whole body MR scanner (VIDA/PRISMA Siemens, Erlangen, Germany) equipped with a 20-channel phased array head coil. Standard imaging protocol included a three-plane scout localizer, T2-weighted, T1-weighted, diffusion-weighted (DWI), and MR perfusion imaging (PWI). Post-contrast T1-weighted images were acquired with the same parameters as pre-contrast acquisition after administration of a standard dose (0.1 mmol/kg) of gadolinium-based contrast agent using an automated power injector.

^{1}H-MRS protocol

^{1}H-MRS studies were performed on the 3T scanners mentioned above. Single-voxel PRESS (Point-RESolved Spectroscopy) method was used with long (135 ms) and short (30 ms) echo time (TE). Within the tumour, the size and location of the voxel were carefully adjusted to include as much of the solid tumour portion as possible, avoiding the inclusion of the scalp, skull base, sinuses, areas of obvious necrosis, cyst, haemorrhage, oedema, calcification, and normal-appearing brain tissue. To optimize the parameters and the magnetic field homogeneity in some patients, manual shimming was performed in order to obtain the narrowest possible water signal (minimum of the full width at half maximum – FWHM – the average value was 11 Hz (values ranged from 7 to 18 Hz depending on the voxel location)). The residual signal of the water was then eliminated from the spectrum.

Data analysis

For the spectroscopic technique, voxels were placed within hyperintense regions on T1-weighted images after contrast administration or T2-weighted FLAIR images (in case of lesions that did not enhance after contrast injection). ^{1}H-MRS data were analysed based on MR Spectro (SyngoVia software v. B20, Siemens, Erlangen, Germany). Signals from contralateral voxels containing normal-appearing brain tissue (NABT) were analysed for comparison. The MR Spectro software allowed fitting of individual metabolite peaks that could be optimised by the user. The following metabolites were evaluated: NAA – 2.02 ppm, creatine – 3.02 ppm, choline – 3.22 ppm, Myo-inositol (mI) – 3.56 ppm and in the next step, their ratio and ratio to contralateral NABT were calculated.

The gliomas were classified into the group of no-choline elevation when the ratio of choline measured within the tumour (Cho_{lesion}) to choline from NABT (Cho_{ref}) was equal to or lower than 1 (Fig. 1).

The calculated metabolite ratios that were calculated are shown in Table II.

Metabolite ratios were calculated for echo time (TE) 135 ms as follows: Cho/Cr, Cho/NAA within the lesion and the ratio of the choline level in the lesion to the choline level in the NABT. All 89 patients included into the study had good quality MR spectra. In 20% of cases (18 of 89) within grade II-III gliomas, choline levels did not increase. Choline levels in these lesions were lower than in contralateral reference voxels ($Cho_{lesion}/Cho_{ref} < 1$). 28% (17 of 74) of patients with brain tumours with lack of choline elevation in ^{1}H-MRS were verified as grade II gliomas, and 7% (1 out of 15) as grade III.

Statistical analysis

Data analysis was performed using the Statistica (v.12) statistical software package. The Shapiro-Wilk test was used to check whether a continuous variable follows a normal distribution. Data were presented as median and quartile ranges. To assess differences among variables, the Mann-Whitney *U* test was used to compare two independent groups. The differences in the group were also shown in the box-plots (Figs. 2-5). The significance level was established at $\alpha = 0.05$.

We would like to increase the number of cases under analysis; however, due to the significantly lower occurrence rate of the described phenomenon in WHO III tumours compared to WHO II, this task is expected to span several more years.

Results

The descriptive statistics of the glial tumour and no-choline elevation tumour groups and the *p*-value of the comparison of these groups were presented in Table III.

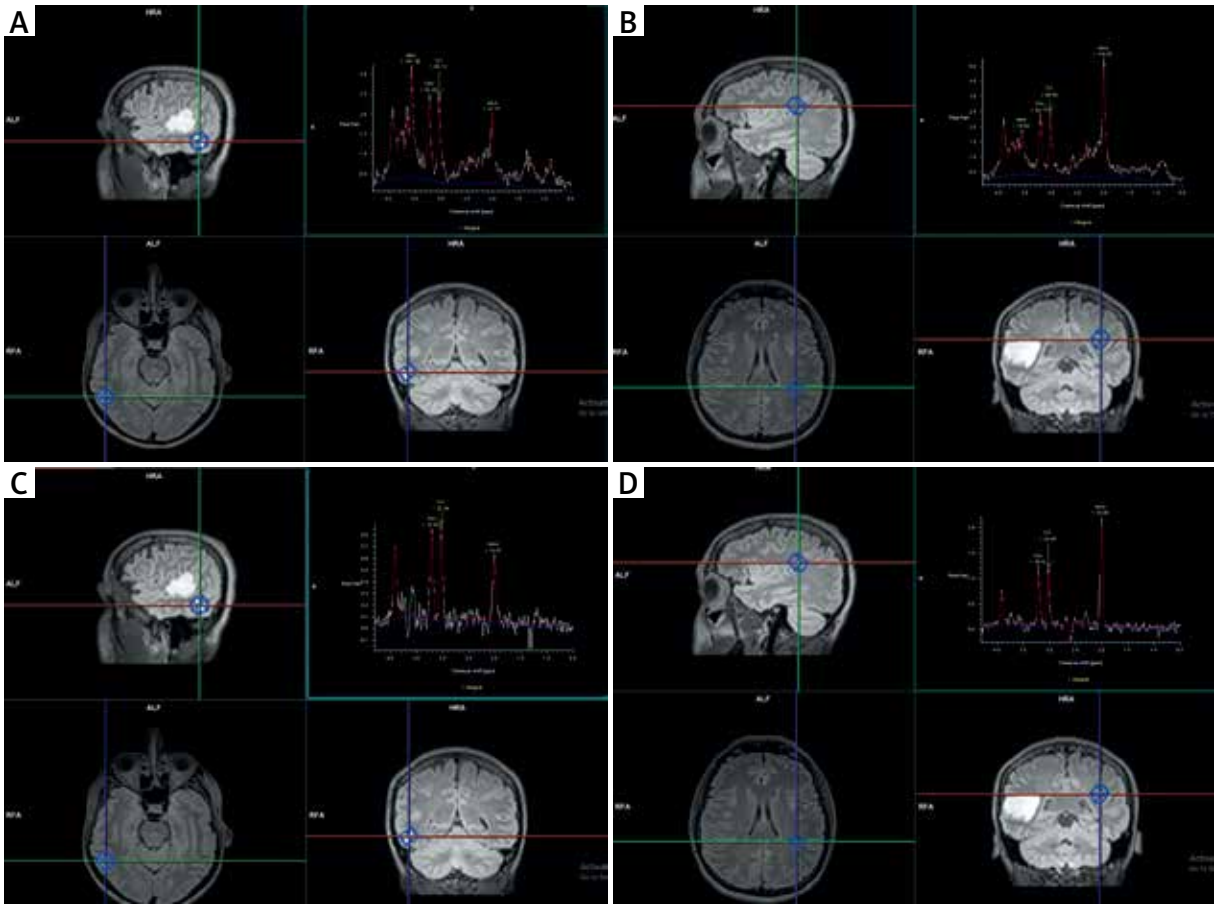

Fig. 1. Example of no-choline elevation in ^{1}H-MRS spectra (patient with verified astrocytoma): **A**) TE = 30 ms, **B**) reference TE = 30 ms, **C**) TE = 135 ms, **D**) reference TE = 135 ms.

Table II. Calculated metabolite ratios obtained with echo time (TE) 30 ms and 135 ms

Metabolite ratio (TE = 135 ms)	Metabolite ratio (TE = 30 ms)
Cho/Cr	mI/Cr
NAA/Cr	mI_{lesion}/mI_{ref}
Cho/NAA	
Cho_{lesion}/Cho_{ref}	
NAA_{lesion}/NAA_{ref}	

Fig. 2. Box-plot of Cho_{lesion}/Cho_{ref} ratios in the no-choline elevation and glial tumour groups.

The Mann-Whitney *U* test was used to verify a hypothesis determining insignificance of differences between analysed variable in 2 groups (the WHO III case was excluded). There were no statistically significant differences in the ratios of the reference values between analysed groups.

The difference between the calculated Cho_{lesion}/Cho_{ref} ratios allowed us to divide analysed cases into two groups – 17 patients were included to the no-choline elevation group and 57 patients were enrolled into the rest of the gliomas.

Significant differences were observed between the ratios of Cho_{lesion}/Cr_{lesion} (p = 0.001) calculated for no-choline elevation and glial tumour groups. The val-

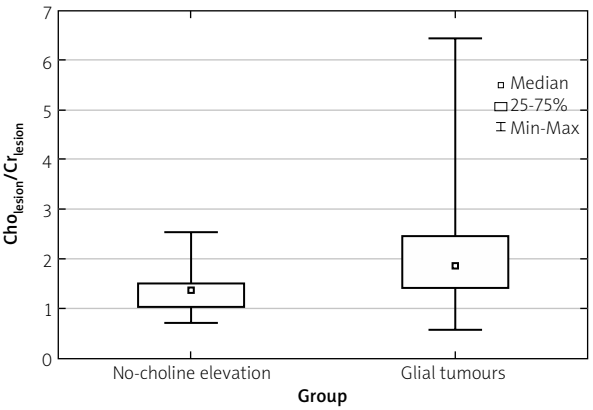

Fig. 3. Box-plot of Cho_{lesion}/Cr_{lesion} ratios in the no-choline elevation and glial tumour groups.

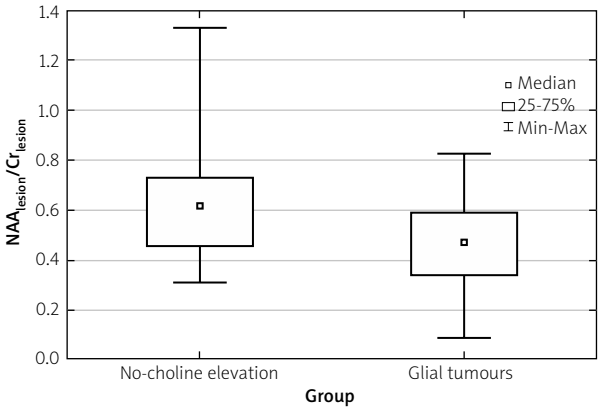

Fig. 4. Box-plot of NAA_{lesion}/Cr_{lesion} ratios in the no-choline elevation and glial tumour groups.

ues computed for the metabolites in no-choline elevation group were significantly lower.

There were significant differences in the NAA_{lesion}/Cr_{lesion} ratio (p = 0.030) between the no-choline elevation group and glial tumour group. Values in the no-choline elevation group were higher. Based on the calculations there was no difference in creatine levels and it should be assumed that this difference resulted from a slightly higher level of NAA in the no-choline elevation group compared to other WHO II glial tumour group.

With consistent data concerning the choline level elevation and slightly lower NAA value, the $Cho_{lesion}/NAA_{lesion}$ ratio was significantly higher in the WHO II glial tumour group compared to the no-choline elevation cases (p < 0.000) (Table IV).

In agreement with earlier calculations, greatest differences were observed in the choline level ratios: the patient in the no-choline elevation group had significantly lower levels of choline in GIII glial tumours than in other GIII cases. Also notable is the markedly lower level of mIns in the group without choline elevation compared to the evident increase in this metabolite

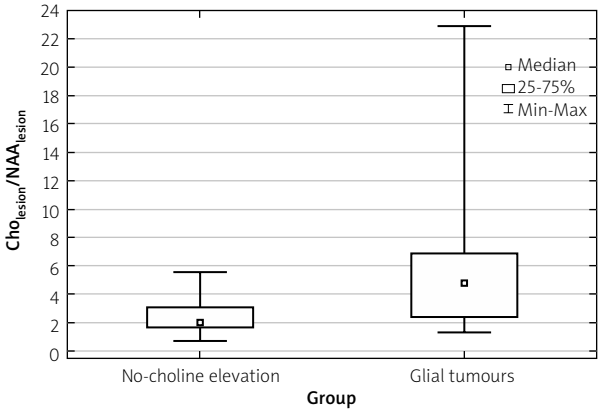

Fig. 5. Box-plot of $Cho_{lesion}/NAA_{lesion}$ ratios in the no-choline elevation and glial tumour groups.

concentration in the standard GIII glial tumour group (which was not observed in the WHO II glial tumour group). Due to the small size of the group – no-choline elevation in GIII glial tumours is only one patient compared to 14 patients with GIII glial tumours, only a case

Table III. Descriptive statistics of the glial tumour and no-choline elevation tumour WHO II groups

Metabolite ratio, Echo time	Glial tumour group with no-choline elevation group (WHO II); n = 17 Median [Q1-Q3]	Glial tumour group (elevated choline level) (WHO II), n = 57 Median [Q1-Q3]	z-value
Cho_{lesion}/Cho_{ref} TE = 135 ms	0.81 [0.71-0.92]	1.46 [1.24-1.81]	< 0.000
Cr_{lesion}/Cr_{ref} TE = 135 ms	0.79 [0.66-1.04]	0.89 [0.68-1.11]	0.456
NAA_{lesion}/NAA_{ref} TE = 135 ms	0.40 [0.27-0.62]	0.31 [0.19-0.48]	0.217
Cho_{lesion}/Cr_{lesion} TE = 135 ms	1.37 [1.02-1.51]	1.84 [1.41-2.45]	0.001
NAA_{lesion}/Cr_{lesion} TE = 135 ms	0.61 [0.46-0.73]	0.47 [0.34-0.59]	0.030
$Cho_{lesion}/NAA_{lesion}$ TE = 135 ms	1.92 [1.67-3.10]	4.75 [2.43-6.89]	< 0.000
$mIns_{lesion}/mIns_{ref}$ TE = 30 ms	1.15 [0.9-1.38]	1.27 [0.91-1.60]	0.544
$mIns_{lesion}/Cr_{lesion}$ TE = 30 ms	2.04 [1.62-2.79]	2.14 [1.46-2.97]	0.995

report is possible; other statistical calculations will be conceivable once the group size is increased.

Discussion

Until now, histopathology has remained the gold standard in neuro-oncology as in all oncology [13]. A stereotactic biopsy or an open biopsy are usually the best way to verify the suspicious lesion [3], however, in some cases this procedure could not be performed. ^{1}H-MRS is one of the non-invasive methods of differential diagnosis of intra-axial lesions [15,12,21]. In this study, we retrospectively assessed ^{1}H-MRS in a group of grade II–III gliomas. It was detected that some of the gliomas showed no choline level elevation and we tried to estimate the frequency of that abnormality. There could be several reasons why some gliomas do not show elevation in choline levels on ^{1}H-MRS, including tumour localization, partial volume effect, or other yet-to-be-understood reasons. Generally, ^{1}H-MRS is useful for diagnosis and monitoring of gliomas, the lack of elevated choline levels raises doubts about the neoplastic nature of the lesion, however, the method has some well-known limitations. First, there is a lack of standardized analysis, differences in acquisition parameters, field strength, and limited availability. Also problematic are tissue heterogeneity, voxel size and localization that may affect the local concentration of metabolites. Despite these facts, therapy planning can benefit from ^{1}H-MRS and spectra analysis needs to be taken into consideration in the preoperative differential evaluation of brain masses.

Several studies have focused on understanding metabolic changes in gliomas revealing that Cho/Cr and Cho/NAA are the two ratios that have been mainly used for the purpose of glioma staging. In some articles there were presented calculations of both ratios and it was shown that it may be useful in glioma grading [6,8,9]; nonetheless, some studies measured only the Cho/Cr ratio as the main ratio, and we followed that mindset. An increased ratio of Cho/Cr metabolites is understood as a result of increased cellularity and higher membrane turnover in brain tumours, which was used to differentiate the histological grade based on the degree of Cho/Cr elevation [1,6,19].

In the present study, we noticed that a certain part of the gliomas demonstrated the absence of Cho/Cr and Cho_{lesion}/Cho_{ref} elevation. To date, few papers describing no choline elevation in gliomas were published [4,10,16], so in our opinion more studies are needed regarding the absence of choline elevation in grade II-III gliomas. The exact reason for the lack of choline elevation in gliomas is not known; it could be due to several factors, however, it is supposed that the presence of relatively less neoplastic cells in the active division phase of the cell cycle in these gliomas could cause not increase choline levels [10]. Another possible explanation is that the tumour has a lower cell density or a slower growth rate, which would result in lower levels of choline. There is also a possibility that the tumour has a different metabolic profile or molecular subtype that does not produce high levels of choline. Furthermore, the heterogeneity of gliomas, even within the same grade, could contribute to the lack of choline elevation. Some regions of the tumour may exhibit high levels of choline, whereas other regions may not because of different molecular characteristics. It is possible that the gliomas without elevation of choline levels were heterogeneous tumours with areas of low choline levels. This variability could be influenced by a variety of factors, such as the tumour microenvironment, genetic mutations, or metabolic changes. It is also important to consider technical factors that could affect choline measurements in MRS. For example, partial volume effects, variability in acquisition parameters, and variations in the size and shape of the voxel could all impact choline measurements. The location of the tumour within the brain can also affect the choline levels detected in ^{1}H-MRS. For example, if the tumour is located in an area of the brain with a high lipid content, such as the occipital lobe, the choline peak may be obscured by the lipid signal.

In our study, 89 patients met our specific criteria for inclusion cases of grade II and III gliomas. These criteria ensured the selection of patients with supratentorial tumour localization, excluding cases with ependymomas, and ensuring adequate tumour dimensions for high-quality MRS voxel placement. The SNR in the acquired spectra was carefully maintained at a minimum of 2. Furthermore, all patients in the study received a histologically confirmed diagnosis of grade II or III glioma, and cases with poor-quality MRS spectra or lacking reliable histopathological verification were excluded. The placement of the MRS voxel within the solid part of the tumour, avoiding cystic/necrotic areas and other confounding factors, was meticulously carried out. The reference voxel was positioned contralaterally in the normal brain tissue. Surgical interventions were performed to either completely resect the tumour or partially resect it when located near sensitive brain regions. Interestingly, in 20% of cases within the grade II-III glioma group, choline levels did not increase, as evidenced by the Cho_{lesion}/Cho_{ref} ratio being equal to or lower than 1. Within this subgroup, 28% were identified as grade II gliomas and 7% as grade III. This division into two groups, the no-choline elevation and the remaining gliomas, revealed significant differences in Cho_{lesion}/Cr_{lesion} and NAA_{lesion}/Cr_{lesion} ratios. The values of these metabolites were notably lower in the no-cho-

Table IV. Descriptive statistics of the glial tumour and no-choline elevation tumour WHO III groups

Metabolite ratio, Echo time	No-choline elevation group (WHO III), n = 1 Value	Glial tumours group (elevated choline level) (WHO III), n = 14 Median [Q1-Q3]
Cho_{lesion}/Cho_{ref} TE = 135 ms	0.99	2.25 [1.77-3.18]
Cr_{lesion}/Cr_{ref} TE = 135 ms	0.77	0.88 [0.72-1.02]
NAA_{lesion}/NAA_{ref} TE = 135 ms	0.43	0.20 [`0.12-0.30]
Cho_{lesion}/Cr_{lesion} TE = 135 ms	1.40	2.88 [2.10-4.15]
NAA_{lesion}/Cr_{lesion} TE = 135 ms	0.67	0.31 [0.19-0.38]
$Cho_{lesion}/NAA_{lesion}$ TE = 135 ms	2.08	8.89 [7.13-22.51]
$mIns_{lesion}/mIns_{ref}$ TE = 30 ms	0.31	1.41 [1.12-1.95]
$mIns_{lesion}/Cr_{lesion}$ TE = 30 ms	0.44	2.46 [1.93-2.92]

line elevation group, with the $Cho_{lesion}/NAA_{lesion}$ ratio being significantly higher in WHO II glioma patients compared to those with no choline elevation. Additionally, we observed remarkable variations in choline and myo-inositol (mIns) levels. Patients in the no-choline elevation group exhibited significantly lower choline levels in grade III gliomas compared to the standard group, while mIns levels were notably lower in the no-choline elevation group compared to the evident increase in mIns concentration in the standard grade III glioma group. However, due to the limited size of the no-choline elevation group, further statistical analysis awaits a larger sample size.

In general, the lack of choline elevation in certain gliomas is a complex phenomenon that could be influenced by a variety of biological and technical factors. More research is needed to better understand the mechanisms underlying this phenomenon and develop more accurate and reliable diagnostic methods for gliomas. In summary, this study highlights the importance of using multiple imaging techniques to accurately assess the grade of glioma and avoid the risk of misdiagnosis or exclusion of lower-grade tumours. Magnetic resonance spectroscopy can be a valuable tool in this process but should be used in conjunction with other measures to provide a comprehensive diagnosis.

Conclusions

Gliomas of each grade have some specific features in MRS that can be used for improvement of the diagnostic value of conventional MRI in non-invasive assessment of the glioma grade. In the current study, the results demonstrated the possibility of lack of choline elevation in patients with grade II-III gliomas. It is very important in the differential diagnosis of brain lesions to rely on conventional diffusion and perfusion magnetic resonance imaging and other spectroscopy measurements such as Cho/NAA and mI/Cr ratios, to avoid the risk of excluding grade II-III glioma if there is no Cho/Cr elevation. In some cases, choline could be not elevated in grade II-III gliomas. The lack of elevated choline levels does not exclude the neoplastic lesion.

Therefore, while the presence or absence of choline elevation is an important factor to consider in the diagnosis and grading of gliomas, it is only one piece of the puzzle and should be evaluated in conjunction with other clinical and imaging factors.

Disclosure

The authors report no conflict of interest.

References

1. Brandão LA, Castillo M. Adult brain tumors: Clinical applications of magnetic resonance spectroscopy. Magn Reson Imaging Clin N Am 2016; 24: 781-809.
2. Brandner S, Jaunmuktane Z. Neurological update: gliomas and other primary brain tumours in adults. J Neurol 2018; 265: 717-727.
3. Bulik M, Jancalek R, Vanicek J, Skoch A, Mechl M. Potential of MR spectroscopy for assessment of glioma grading. Clin Neurol Neurosurg 2013; 115: 146-153.
4. Chawla S, Lee SC, Mohan S, Wang S, Nasrallah M, Vossough A, Krejza J, Melhem ER, Nabavizadeh SA. Lack of choline elevation on proton magnetic resonance spectroscopy in grade I-III gliomas. Neuroradiol J 2019; 32: 250-258.
5. Ditter P, Hattingen E. MR-Spektroskopie bei Hirntumoren. Radiologe 2017; 57: 450-458.
6. Fountas KN, Kapsalaki EZ, Vogel RL, Fezoulidis I, Robinson JS, Gotsis ED. Noninvasive histologic grading of solid astrocytomas using proton magnetic resonance spectroscopy. Stereotact Funct Neurosurg 2004; 82: 90-97.
7. Izquierdo C, Joubert B, Ducray F. Anaplastic gliomas in adults: an update. Curr Opin Oncol 2017; 29: 434-442.
8. Kousi E, Tsougos I, Tsolaki E, Fountas KN, Theodorou K, Fezoulidis I, Kapsalaki E, Kappas C. Spectroscopic evaluation of glioma grading at 3T: The combined role of short and long TE. Scientific World Journal 2012; 2012: 546171.
9. Liu ZL, Zhou Q, Zeng QS, Li CF, Zhang K. Noninvasive evaluation of cerebral glioma grade by using diffusion-weighted imaging-guided single-voxel proton magnetic resonance spectroscopy. J Int Med Res 2012; 40: 76-84.

10. Londoño A, Castillo M, Armao D, Kwock L, Suzuki K. Unusual MR spectroscopic imaging pattern of an astrocytoma: lack of elevated choline and high myo-inositol and glycine levels. AJNR Am J Neuroradiol 2003; 24: 942-945.
11. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, Ellison DW. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol 2021; 23: 1231-1251.
12. Naser RKA, Hassan AAK, Shabana AM, Omar NN. Role of magnetic resonance spectroscopy in grading of primary brain tumors. Egypt J Radiol Nucl Med 2016; 47: 577-584.
13. Rafique Z, Awan MW, Iqbal S, Usmani NN, Kamal MM, Arshad W, Ahmad M, Mumtaz H, Ahmad S, Hasan M. Diagnostic accuracy of magnetic resonance spectroscopy in predicting the grade of glioma keeping histopathology as the gold standard. Cureus 2022; 14: e22056.
14. Ramli N, Khairy AM, Seow P, Tan LK, Wong JHD, Ganesan D, Rahmat K. Novel application of chemical shift gradient echo in- and opposed-phase sequences in 3 T MRI for the detection of H-MRS visible lipids and grading of glioma. Eur Radiol 2016; 26: 2019-2029.
15. S AJ, Jose T. Role of magnetic resonance spectroscopy in grading of gliomas-a tertiary care centre study. Int J Res Med Sci 2022; 10: 381-387.
16. Saraf-Lavi E, Bowen BC, Pattany PM, Sklar EML, Murdoch JB, Petito CK. Proton MR spectroscopy of gliomatosis cerebri: case report of elevated myoinositol with normal choline levels. AJNR Am J Neuroradiol 2003; 24: 946-951.
17. Suh CH, Kim HS, Jung SC, Choi CG, Kim SJ. 2-Hydroxyglutarate MR spectroscopy for prediction of isocitrate dehydrogenase mutant glioma: a systemic review and meta-analysis using individual patient data. Neuro Oncol 2018; 20: 1573-1583.
18. Vamvakas A, Williams SC, Theodorou K, Kapsalaki E, Fountas K, Kappas C, Vassiou K, Tsougos I. Imaging biomarker analysis of advanced multiparametric MRI for glioma grading. Phys Med 2019; 60: 188-198.
19. Vuori K, Kankaanranta L, Häkkinen AM, Gaily E, Valanne L, Granström ML, Joensuu K, Blomstedt G, Paetau A, Lundbom N. Low-grade gliomas and focal cortical developmental malformations: differentiation with proton MR spectroscopy. Radiology 2004; 230: 703-708.
20. Wei J, Yang G, Hao X, Gu D, Tan Y, Wang X, Dong D, Zhang S, Wang L, Zhang H, Tian J. A multi-sequence and habitat-based MRI radiomics signature for preoperative prediction of MGMT promoter methylation in astrocytomas with prognostic implication. Eur Radiol 2019; 29: 877-888.
21. Weis J, Ring P, Olofsson T, Ortiz-Nieto F, Wikström J. Short echo time MR spectroscopy of brain tumors: grading of cerebral gliomas by correlation analysis of normalized spectral amplitudes. J Magn Reson Imaging 2010; 31: 39-45.