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RESEARCH ARTICLE

INVESTIGATION OF CREATINE LEVELS IN GLIAL TUMORS USING MR SPECTROSCOPY

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ABSTRACT

Purpose: The present study examines the efficacy of Creatine (Cr) levels in tumor identification on single-voxel (SV) and multi-voxel (MV) magnetic resonance spectroscopy (MRS) in patients with glial tumors.
Material and Methods: A retrospective review was made of 20 SV and 20 MV MRS PDF images of patients with a pathological diagnosis of glial tumor. Cr values and metabolite ratios were measured in the tumor and compared with those in the healthy symmetrical brain parenchyma of the contralateral hemisphere.
Results: A significant difference was noted in the (Cho+Cr)/NAA and Cho/NAA ratios on SV MRS between the high-grade tumors and the healthy contralateral hemisphere. On MV MRS, Cho/NAA ratios within the minimum and maximum Cr(h) voxels ($Cho/NAA_{min\text{-}Cr(h)}$, $Cho/NAA_{max\text{-}Cr(h)}$, respectively) were higher in high-grade tumors compared to healthy tissue. ROC analysis showed that the max-Cr(h) metabolite on MV MRS was successful in distinguishing low-grade tumors from high-grade tumors.
Conclusion: Using the minimum and maximum values of Cr as a reference can improve the overall diagnostic accuracy in the diagnosis of glial tumors. Our findings showed that Cr tended to be low in high-grade tumors and further that the max-Cr(h) metabolite may help in the differentiation of glial tumors on MV MRS.

Keywords: Magnetic resonance imaging, spectroscopy, glial tumors, creatine

INTRODUCTION

Glial tumors account for 30% of primary brain tumors and originate from glial cells, which are the support cells in the brain. A positive diagnosis is established by surgery or biopsy and is used to assess prognosis and guide treatment. Depending on the location and grade of the tumor, various treatments, including surgery, radiotherapy and chemotherapy may be administered alone or in combination (1).

Since lesions may develop from regions with different malignancies, the approaches to the examination of heterogeneity in the lesion area are quite important in gliomas (2). Conventional magnetic resonance imaging (MRI) (T1, T2, and contrast-enhanced MRI) is a useful clinical tool for the identification of soft tissue contrast and morphological changes in glioma patients but remains insufficient in image-based tumor grading (2, 3). Besides, the diagnosis and grading of gliomas using conventional MRI may sometimes be unreliable due to the sensitivity of 55–83% in glioma grading (4). As such, alternative MRI approaches are needed for the investigation of tumor

Table 1. Demographic and clinical characteristics of participants

	SV	MV
Age (years / mean ± SD)	53.25 ± 14.61	54.40 ± 14.81
Gender (F/M)	9/11	10/10
Total (n)	20	20
Preop (n)	15	12
Postop (n)	5	8
Grade II	5	6
Grade III	5	2
Grade IV	10	12

F: Female, M: Male, MV: Multi-voxel, SD: Standard deviation SV: Single-voxel

metabolisms and the determination of the tumor grade, and to guide treatment planning.

Magnetic resonance spectroscopy (MRS) is an MRI approach that plays an essential role in determining the type and grade of most brain tumors. Developed for the chemical analysis of normal or pathological tissues at a molecular level, MRS can detect malignant transformations of tumor cells and metabolite concentration changes in their metabolism, thereby being useful in the prediction of tumor type and grade (5, 6). Glial tumors have some specific metabolites depending on their grade, and these metabolites vary in quantity as the tumor grade changes.

Creatine (Cr) is synthesized primarily from amino acids, and mostly in the kidneys and liver. It is transported to the peripheral organs in the blood and is referred to as an energy metabolism marker. Cr is a relatively constant element in the cellular energy metabolism of the brain and is often used as a reference metabolite in the calculation of such metabolite ratios as Cho/Cr and NAA/Cr (7).

Previous studies have identified the same Cr levels in both low-grade and high-grade gliomas (8, 9), while other studies have reported reduced Cr levels in brain tumors (10, 11). Furthermore, the amount of Cr may vary in different parts of the same tumor, with elevated Cr having been identified in hypometabolic areas and reduced Cr in hypermetabolic areas of the same tumor (12). The significance of Cr levels in the differentiation of high- and low-grade gliomas is unclear. If the Cr metabolite at the tumor site is used as an internal standard, differences in metabolite ratios may be difficult to identify, and so tumor-grade predictions may be erroneous. Cr, which provides information about the energy metabolism of glial tumor tissue on MRS, has been reported to vary according to the grade of the tumor in limited studies (7, 13), contradicting the general understanding that the Cr metabolite is stable.

In the present study, we examine the efficacy of Cr levels for tumor identification in single-voxel (SV) and multi-voxel (MV) MRS examinations in patients with glial tumors. We hypothesized that the ratios of Cr metabolite and Cr-related metabolite ratios could differentiate glial tumors from normal tissue.

MATERIAL AND METHODS

Subjects

MRS images of patients who were diagnosed pathologically with glial tumors were analyzed retrospectively. The subjects included 32 patients who had undergone MRS due to a pre-diagnosis of primary brain tumor, or in a follow-up examination following surgical treatment, who had undergone surgical resection, and who were diagnosed pathologically with glial tumors. An SV MRS examination of tumor tissue and healthy symmetrical brain parenchyma of the contralateral hemisphere was performed for comparison purposes in 20 patients, and an MV MRS examination was performed on 20 patients. The eight common patients in the two patient groups underwent both SV and MV examinations. Demographic and clinical data of the patients are presented in Table 1. All subjects provided written informed consent and the study protocol was approved by the Dokuz Eylül University

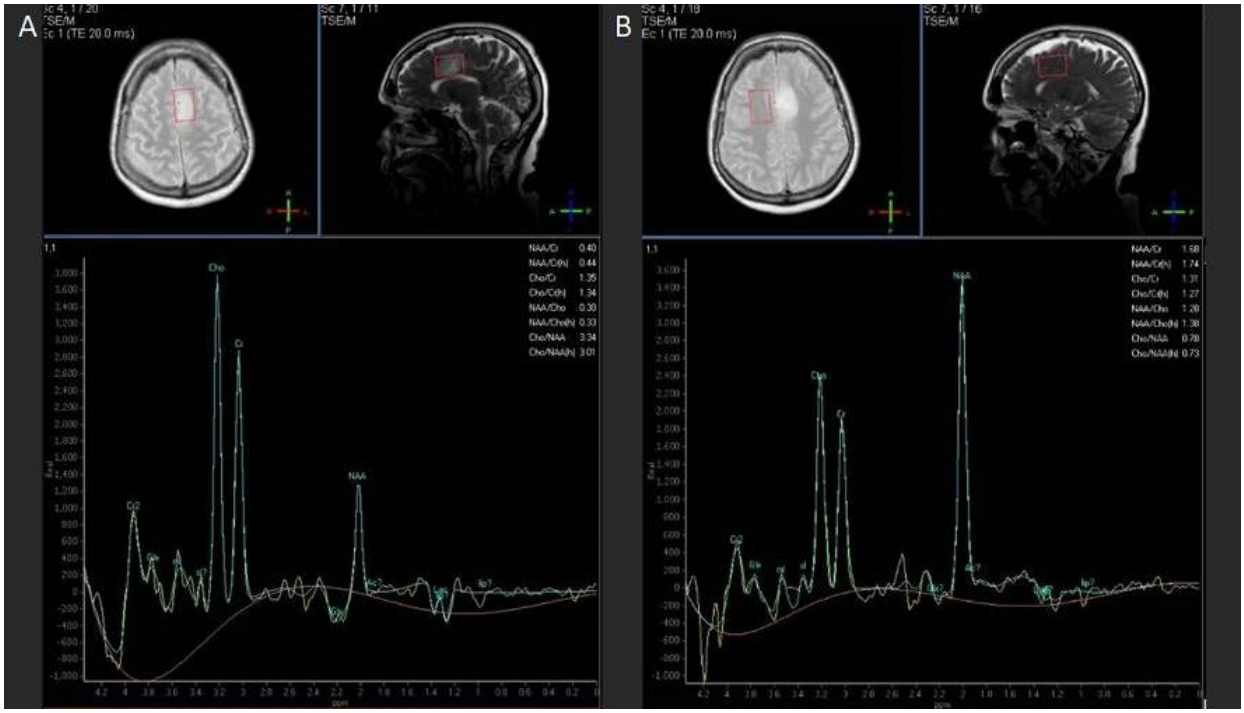

Figure 1. A 52-year-old patient with a lesion located in the right frontoparietal region. Metabolite measurements from A) tumor tissue and B) symmetric, healthy brain parenchyma for comparison with tumor tissue in MV MRS examination.

Non-invasive Research Ethical Committee (decision date: February 2, 2016, decision no: 2016/04-10).

MR Imaging Acquisition

MR imaging was performed using the 1.5 Tesla Achieva MR scanner (Philips Medical Systems, Best, The Netherlands) in the Radiology Department of Dokuz Eylül University. First, TSE T2-weighted images (TR: 6000 ms, TE: 120 ms) were acquired with a 5-mm section thickness in the axial-sagittal-coronal planes for voxel localization. Then, SV (TE: 144 ms) and 2-cm thick single-section MV (TE: 288 ms) MRS scans were acquired using the PRESS sequence. For SV MRS, voxels with the smallest size of 2 cm^3 and the largest size of 9 cm^3 were positioned within the tumor tissue and the corresponding contralateral healthy brain tissue (Figure 1). On the other hand, the voxels were positioned to include the tumor and the contralateral hemisphere for MV MRS (Figure 2).

MRS Analysis

In the SV MRS examination the Cr height (h), Cr area (a), Cho/NAA, and (Cho+Cr)/NAA values, calculated automatically by the MR unit's computer, were compared with the values in the healthy contralateral hemisphere. In the MV MRS examination, the voxels were examined in the central tumor tissue and the areas with infiltration of the surrounding tissue, and the two voxels, within which the minimum and maximum height of the Cr (Cr(h)) metabolite were measured, were compared with the reference "Cr(h)" values within the healthy contralateral parenchyma. Furthermore, the Cho/NAA ratios of the tumor tissue within the minimum and maximum Cr(h) voxels ($Cho/NAA_{min\text{-}Cr(h)}$, $Cho/NAA_{max\text{-}Cr(h)}$, respectively) were compared with the Cho/NAA ratios of the healthy contralateral side.

Statistical Analysis

The SPSS version 24.0 (Armonk, NY: IBM Corp.) was used for all statistical analyses. Non-parametric tests were used as the sample was small and the data were not normally distributed. The tumors were classified pathologically as low grade (DII) and high grade (DIII-DIV). A Mann-Whitney U test or a Wilcoxon test with Bonferroni correction were used to assess the differences in metabolites and metabolite ratios between high-grade and low-grade tumors in both groups. The optimal cut-off values for the metabolite ratios and the area under the curve (AUC) (95% confidence interval (CI)) were calculated using a receiver-operating characteristic curve (ROC) analysis to assess the ability to identify the tumor

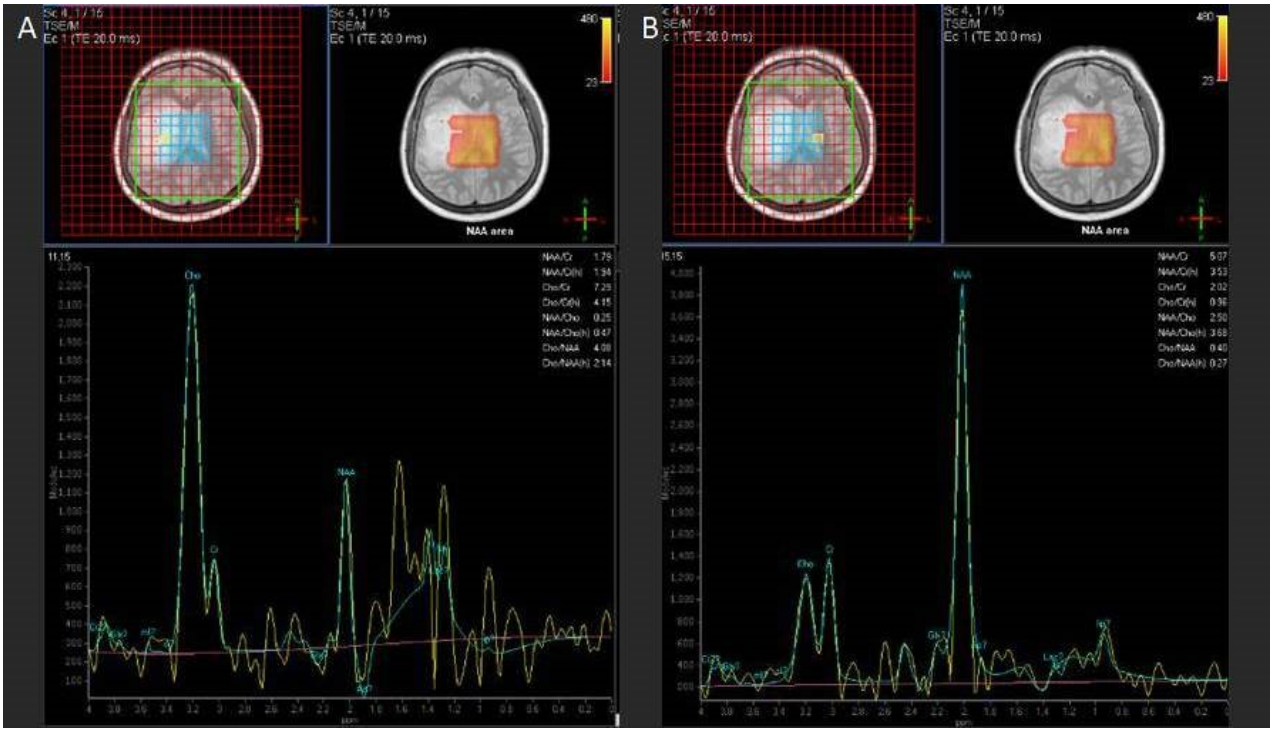

Figure 2. A 67-year-old patient with a lesion adjacent to the falx in the left frontal lobe. Metabolite measurements from A) tumor tissue and B) healthy contralateral frontal lobe for comparison with tumor tissue in SV MRS examination.

grades. A p-value of <0.05 was considered statistically significant.

RESULTS

The SV MRS examination revealed no difference between low-grade and high-grade tumors in terms of Cr levels or metabolite ratios. For the low-grade tumors, the (Cho+Cr)/NAA (p=0.043) and Cho/NAA (p=0.080) ratios were slightly higher in the low-grade group than in the normal healthy contralateral hemisphere but these differences were not statistically significant after Bonferroni correction. Also, there was no difference between low-grade tumors and normal tissue in terms of Cr(a) and Cr(h). For high-grade tumors, the (Cho+Cr)/NAA (p=0.001) and Cho/NAA (p=0.001) ratios in the tumor tissue and normal tissue differed. Although Cr(a) was lower in the high-grade group than in the normal tissue, this difference was not statistically significant after Bonferroni correction (p=0.047). The comparison of high-grade and low-grade tumors in the SV MRS examination is presented in Table 2.

The MV MRS examination revealed a difference only in max-Cr(h) (p=0.035) between low-grade and high-grade tumors, but this did not survive the Bonferroni correction (Table 3). For low-grade tumors, no statistical difference was found in the measurements of the tumor and the healthy contralateral hemisphere. For high-grade tumors, the $Cho/NAA_{min\text{-}Cr(h)}$ (p=0.001) and $Cho/NAA_{max\text{-}Cr(h)}$ (p=0.001) values between the tumor tissue and normal tissue were different (Table 4).

To assess the ability of MRS to differentiate between tumor tissue and normal healthy tissue, cut-off values for metabolites/metabolite ratios were calculated with a ROC curve analysis. On MV MRS, $Cho/NAA_{min\text{-}Cr(h)}$ ratio had the highest with an AUC value of 1.000 [95% CI: 1.000–1.000] and a cut-off value of 1.21 (sensitivity = 100%, specificity = 100%) for differentiating tumor tissue from normal tissue in high-grade tumors. The AUC, sensitivity, and specificity values of ROC curve analysis are presented in Table 5.

While the ROC curve analysis failed to differentiate between low-grade and high-grade tumors in the SV MRS examination, the MV MRS examination revealed that only max-Cr(h) had an AUC value of 0.804 [95% CI: (0.600–1.000)] and a cut-off value of 2212.5 (sensitivity = 83%, specificity = 64%).

DISCUSSION

In this study, we investigated the role of Cr metabolite in the differentiation of tumor tissue from normal tissue, and its ability to identify tumor grade in

Table 2. Comparison of measurements made from tumor and normal parenchyma in cases with low and high-grade tumors in SV MRS examination

Metabolites	Localization	Low Grade (grade II)			High Grade (grade III-IV)			Mann Whitney p-value
		Mean ± SD	Range	p-value[a]	Mean ± SD	Range	p-value[a]	
Cr(a)	Tumor	91.0 ± 68.9	55.0 – 214.0	0.893	100.3 ± 62.3	35.0 – 237.0	0.047	0.735
	Normal	88.6 ± 47.1	40.0 – 154.0		132.7 ± 70.9	56.0 – 283.0		0.168
Cr(h)	Tumor	1207.2 ± 1019.2	598.0 – 3022.0	0.893	1133.2 ± 661.2	355.0 – 2173.0	0.281	0.866
	Normal	1121.4 ± 644.3	407.0 – 1983.0		1274.3 ± 562.6	706.0 – 2839.0		0.672
(Cho+Cr)/NAA	Tumor	3.8 ± 2.0	1.7 – 6.0	0.043	6.5 ± 4.9	1.5 – 17.5	***0.001***	0.497
	Normal	1.2 ± 0.2	1.0 – 1.5		1.4 ± 0.5	0.7 – 2.5		0.395
Cho/NAA	Tumor	2.5 ± 1.6	0.6 – 4.5	0.080	4.8 ± 3.8	1.1 – 13.5	***0.001***	0.266
	Normal	0.7 ± 0.2	0.5 – 0.9		0.8 ± 0.3	0.3 – 1.4		0.445

a: Area, Cho: Choline, Cr: Creatine, h: Height, NAA: N-acetyl aspartate

A: Wilcoxon signed ranks test.

Statistically significant results that survived Bonferroni correction for multiple comparisons (p≤0.0125) are highlighted in bold and italics.

Table 3. Comparison of measurements made from tumor and normal parenchyma in cases with low and high-grade tumors in MV MRS examination

	Low Grade (grade II)		High Grade (grade III-IV)		
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**p-value**
Cr (N)	2020.7 ± 630.6	1010.0 – 2880.0	1516.2 ± 726.5	386.0 – 2530.0	0.216
Cho/NAA (N)	0.2 ± 0.1	0.1 – 0.4	0.3 ± 0.2	0.1 – 0.9	0.385
min-Cr(h) (T)	1793.5 ± 963.3	650.0 – 3062.0	1459.6 ± 649.9	260.0 – 2330.0	0.117
Cho/NAA$_{min\text{-}Cr(h)}$ (T)	2.0 ± 1.2	0.7 – 3.7	3.8 ± 2.7	1.6 – 9.9	0.099
max-Cr(h) (T)	3277.0 ± 1065.9	2040.0 – 4742.0	2046.5 ± 909.8	674.0 – 4224.0	0.035
Cho/NAA$_{max\text{-}Cr(h)}$ (T)	1.5 ± 1.2	0.3 – 3.4	2.8 ± 2.2	0.3 – 7.4	0.248

Cho: Choline, Cr: Creatine, h: height, NAA: N-acetyl aspartate, N: Measurements from normal healthy parenchyma tissue, T: Measurements from tumor tissue
Statistically significant results that survived Bonferroni correction for multiple comparisons (p≤0.0125) are highlighted in bold and italics.

Table 4. Comparison of measurements made from tumor and normal parenchyma in MV MRS examination

	Low Grade	High Grade
	p-value	**p-value**
min-Cr(h)	0.600	0.198
max-Cr(h)	0.028	0.084
Cho/NAA$_{min\text{-}Cr(h)}$	0.028	**0.001***
Cho/NAA$_{max\text{-}Cr(h)}$	0.028	**0.001***

Cho: Choline, Cr: Creatine, h: height, NAA: N-acetyl aspartate. Statistically significant results that survived Bonferroni correction for multiple comparisons (p≤0.0125) are highlighted in bold and italics.

Table 5. Sensitivity, specificity, and AUC values of metabolite ratios for differentiation tumor from normal parenchyma in high-grade tumors.

		Cutoff	Sensitivity %	Specificity %	AUC ± SE
SV	**(Cho+Cr)/NAA**	2.59	93	100	0.973 ± 0.028
	Cho/NAA	1.73	93	100	0.985 ± 0.017
MV	**Cho/NAA$_{min\text{-}Cr(h)}$**	1.21	100	100	1.000 ± 0.000
	Cho/NAA$_{max\text{-}Cr(h)}$	0.57	93	93	0.959 ± 0.035

AUC: Area under the curve, Cho: Choline, Cr: Creatine, MV: Multi-voxel, NAA: N-acetyl aspartate, SV: Single-voxel

patients with glial tumors on SV and MV MRS. In the MRS examinations of the patients, measurements were made in the tumor and the corresponding symmetrical healthy brain parenchyma of the contralateral hemisphere to compare. The Cho/NAA and (Cho+Cr)/NAA ratios were significantly higher in the high-grade tumors than in the healthy contralateral hemisphere on SV MRS. On MV MRS, Cho/NAA$_{min\text{-}Cr(h)}$ and Cho/NAA$_{max\text{-}Cr(h)}$ ratios were higher in high-grade tumors compared to healthy tissue. ROC analysis showed that only the max-Cr(h) metabolite was successful in differentiating high-grade tumors from low-grade tumors on MV MRS in accordance with our hypothesis. Moreover, the Cho/NAA$_{min\text{-}Cr(h)}$ ratio has the highest diagnostic performance to differentiate tumors from healthy tissue in high-grade tumors.

There is a lack of consensus in the literature on the level of Cr in glioma tumors. MRS studies found the amount of Cr metabolite to be low in high-grade glial tumors (14). The amount of Cr shows that its synthesis from astroglia increases as a reaction to the infiltrative growth of tumor cells (15). Xiaojuan et al. found that the Cr level changed in different areas of the same lesion, with a decrease in some areas of the tumor and an increase in others (16). Our study identified different amounts of Cr, which is used as a constant in ratios such as Cho/NAA and (Cho+Cr)/NAA, within different voxels on MV MRS in tumor tissue and healthy brain parenchyma. We found a trend for a lower Cr(h) in high-grade tumors on MV MRS. Moreover, the ROC analysis revealed the Cho/NAA ratio within the voxels containing maximum Cr(h) had higher diagnostic accuracy than those in high-grade tumors. Therefore, this finding suggests that using minimum and maximum Cr(h) as a reference may play an important role in diagnostic assessment.

It is very important to identify tumor grade before planning effective treatment. Non-invasive glioma grading is still considered challenging, despite its important role in the prognosis and management of patients (8). MRS is being used increasingly as a non-invasive method for the detection and grading of brain tumors, and the diagnostic accuracy in tumor grading can thus be further improved (8, 17). Previous studies have demonstrated the potential of MRS to differentiate between low-grade and high-grade gliomas, and have made frequent use of the Cho/NAA ratio to identify the glioma grade (3). This seems to be related to the elevated Cho, which is caused by an increase in cell membrane turnover due to tumor cell proliferation and reduced NAA values due to the tumor cells pushing and replacing the neuronal structures and damaging the neurons. Hsu et al. reported the (Cho+Cr)/NAA ratio to be the most significant predictive value for the differentiation of glial tumors of different grades when compared to other metabolites (18). Our study could not make any grade differentiation for Cho/NAA or (Cho+Cr)/NAA metabolite ratios, although the max-Cr(h) metabolite tended to be lower in high-grade glial tumors on MV MRS, but did not survive a Bonferroni correction. On the other hand, the ROC analysis revealed the max-Cr(h) to be successful in differentiating between low-grade and high-grade gliomas, with a sensitivity of 83% and a specificity of 64%. These findings suggest that Cr is increased in tumor tissue before the Cho/NAA increase and the tumor grade increase. However, given the limited data further studies are needed to confirm the efficacy of the Cr metabolite.

A potential limitation of our study is the small number of cases with low-grade tumors, thus, the results of

this study should be interpreted as preliminary. Therefore, the study findings could be improved upon with further studies involving larger patient groups.

CONCLUSION

In conclusion, in the present study, Cho/NAA metabolite ratio within the voxels containing the minimum Cr(h) on MV MRS was found to have high diagnostic performance in differentiating between tumor tissue and normal tissue in high-grade tumors. Moreover, the max-Cr(h) metabolite on MV MRS was shown to have good diagnostic performance in differentiating between high-grade and low-grade tumors. We, therefore, suggest that using minimum and maximum Cr(h) as a reference on MV MRS might improve the overall diagnostic accuracy in the diagnosis of glial tumors.

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Author contribution: Conception and design: NM and EA; Supervision: EA; Data collection and processing: NM, SE, EA; Analysis: NM, BÇ; Literature review: NM, BÇ; Writing: NM, BÇ; Critical review: SE, EA.
Conflict of interests: No conflict of interest was declared by the authors.
Ethical approval: Approval for the study was received from Non-Invasive Research Ethics Board of Dokuz Eylül University (decision no: 2016/04-10, date: 11.02.2016).
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