Ref: Ro J Neurol. 2022;21(3)
DOI: 10.37897/RJN.2022.3.3

Comparison of MRS imaging tumor index among high and low grade intra axial brain tumor with histology

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ABSTRACT

Aim. The aim of this study was to compare the MRS imaging tumor index among high-grade and low-grade intra axial brain tumor with histology.

Patients and methods. This prospective observational study consisted of 30 patients who underwent surgery for intra-axial brain glioma in the Department of Neurosurgery, Government Medical College, Thiruvananthapuram in a 1 year duration. Inferences were drawn based on MRI scans of brain (plain + contrast) with MRS and a histological examination.

Results. The normalized area values of 3 metabolite resonances, namely N-Acetyl Aspartate (NAA), Choline (Cho) and Creatinine (Cr), between low grade glioma and high-grade glioma were found to be statistically significant ($p<0.05$). Significant difference was also present among Cho/NAA and Cho/Cr in differentiating low-grade from high-grade glioma.

Conclusion. This study has shown that Cho/NAA and Cho/Cr ratios are reliable determinants of the tumor grade with good sensitivity and specificity. Thus, Cho/NAA and Cho/Cr ratios of MRS maybe used as a diagnostic tool in differentiating low grade gliomas from high grade gliomas.

Keywords: high-grade glioma, low-grade glioma, magnetic resonance spectroscopy (MRS), Choline (Cho), Creatinine (Cr), N-Acetyl Aspartate (NAA)

INTRODUCTION

The central nervous system (CNS) neoplasms are rare and constitute about 1 – 2% of all neoplastic lesions [1]. In India, they constitute about 1.9% of all tumors [2]. World Health Organization (WHO) has classified brain tumors into various groups based on their histopathological features and cells of their origin [3]. Tumors of neuroepithelial origin are most common primary neoplasm of brain, of which gliomas constitute the largest subgroup. Glial cells constitute around 70% of the total cell population in central nervous system, making gliomas the most common group of intracranial neoplasms. They consist of a heterogeneous variety of tumors, with varying grades of malignancy, and can be broadly classified as macro- and microglia [4,5]. Virchow, was the first one to recognize the glial origin of this tumor and coined the term "Glioma" 1863 [6]. Gliomas constitute 35–50% of all intracranial neoplasms. The incidence of gliomas among intracranial tumors in various Indian neurosurgical series is similar to that in the rest of the world [7].

Microglia and astrocytes, while normally inactive, often get activated in the event of an injury or disease and play a role in the pathology of neurological conditions [8-10]. Gliomas are considered to be tumors of neuroepithelial origin and are presently categorized on the basis of their morphological appearances into: astrocytic, oligodendroglial, ependymal and choroid plexus tumors.

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Article history:
Received: 6 September 2022
Accepted: 20 September 2022

DIAGNOSTIC NEUROIMAGING FOR GLIOMAS

Magnetic resonance imaging (MRI) of LGGs demonstrates lesions that are isointense/hypointense on T1-weighted images, homogeneously hyperintense on T2-weighted images, and do not enhance with contrast administration. Some characteristics of LGGs are shared with high-grade gliomas (HGGs), with the exception of contrast enhancement, which is usually, but not always, seen in more aggressive grades of HGG [11]. Calcifications can be detected in around 20% of lesions and appear as distinct hyperintense foci on T1-weighted images and hypointense foci on T2-weighted images [12]. Vasogenic edema and necrosis are not typically seen in LGGs, owing to their slow growth rate. Even though the MRI provides excellent soft tissue contrast, it lacks the sensitivity and specificity to identify the grade and the type of the tumour. This pattern of MRI finding may partly be due to Gadolinium-enhanced necrosis which can be misinterpreted as a tumour, and partly to the fact that a tumour, edema and nonspecific treatment effects are almost indistinguishable in hypointense regions on T2-weighted images [13,14]. These issues can be overcome by the development of new imaging modalities that highlight functional or metabolic properties of the tumor. Advanced MRI techniques, inclusive of MR spectroscopy, were used to differentiate glioma grades and key LGG metabolic mutations, such as those of the isocitrate dehydrogenase 1 (*IDH1*) gene.

Magnetic Resonance Spectroscopy

Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) are based on the principle of Nuclear Magnetic Resonance (NMR). While theoretically, any tissue of the human body can be subjected to MRS, the brain continues to remain the organ of primary interest and focus of clinical MRS studies owing to its homogeneity, easy accessibility of MRS and minimal motion artefacts. MRS is a non-invasive diagnostic technique that provides biochemical insignia of the pathophysiology within the brain. Neurospectroscopy, i.e. MRS of the brain, aids in the diagnosis of both, systemic as well as primary brain disorders that affect the CNS [15].

Key metabolites and their significance

MRS products are labelled in parts per million (ppm) and are represented as a spectrum of resonances (peaks) dispersed along the x-axis, while the amplitudes of these resonances are recorded on the y-axis with the help of an arbitrary scale. In the brain MRS, the peaks of significance include N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myo-inositol (ml), lipids (Lip), lactate (Lac), amino acids, and glutamine and glutamate (Glx) [16-23].

N-acetyl Aspartate (NAA)

N-acetyl aspartate (NAA) shows a highly prominent resonance/peak with its highest peak at 2.02 ppm. Although its precise physiological function and characteristics are unknown, NAA is regarded as a marker for neuronal and axonal integrity. Reduced levels of NAA are often associated with neuronal damage or loss and are also seen to occur with various insults to the brain tissue [16,17,20,23,24,25]. During the development and maturation of the brain in infancy, a gradual and progressive rise in NAA is seen physiologically [16].

Choline (Cho)

The sum of the choline-containing substances, including phosphocholine, phosphatidylcholine and glycerophosphocholine is represented by the choline (Cho) peak, which is visible at 3.2 ppm [17,20]. Choline therefore, not only is a membrane turnover marker, but is also representative of cell membrane constituents [16,17,19,20,23,25].

Raised choline peaks are observed in medical conditions that cause an increase in cell number, increased membrane synthesis or breakdown [16,20,22,25] such as demyelination and malignancies.

Creatine (Cr)

Creatine (Cr), and phosphocreatine (PCr) achieve their peak at 3.0 ppm, making creatine an important marker of brain energy metabolism [16,17,19,20,25]. It is commonly used as an internal standard and is fairly stable [16,17,19,20,25]. Fluctuation in creatine levels are observed during gradual loss of creatine along with other key metabolites as observed in tissue death or necrosis. In craniocerebral trauma, the increase in Cr manifests as a hyperosmolar response, whereas, in rare congenital cases of creatine deficiency, it is absent [16,22].

In this study, we seek to compare the MRS imaging index among high-grade and low-grade intra axial brain tumors along with histology.

METHODOLOGY

This observational study comprised of 30 patients who underwent surgery for intra-axial brain glioma, admitted in the Department of Neurosurgery, Government Medical College Thiruvananthapuram from 1st April 2017 to 31st March 2018. This study included selection of patients on a prospective basis. Patients with an extra axial brain tumor and those patients unwilling for the study were excluded. All details of the patients were collected at the time of MRS and after the collection of biopsy.

Management of Glioma mainly depends upon the grading of tumor at the time of diagnosis. Tumor grading is based on histopathological, genetic, and biochemical factors in brain neoplasms. These factors are of interest owing to their prognostic importance due to which they have the potential to guide and influence clinical management decisions.

Detailed clinical history and physical examination of all patients was done followed by an MRI scan of the brain (plain + contrast) with MRS, including [N-acetyl aspartate (NAA), choline (Cho), creatine (Cr)]. Data was entered in excel sheets and analyzed using SPSS software. All qualitative variables were expressed as proportion and quantitative variables in mean and SD. The mean ratios of NAA, Cr and Choline were compared between tumor grade using student t-test and ANOVA. P Value of <0.05 was considered statistically significant.

OBSERVATIONS & RESULTS

Among the 30 patients of Glioma analyzed at our institution, the youngest patient was aged 9 years old, while the oldest patient was aged 69 years old. The mean presentation was 41.13 years. Maximum number of patients were found in the age group of 40 – 51 years (n = 9).

TABLE 1. The age-wise sex distribution of study population

Age	Male		Female		Total	
	N	%	N	%	N	%
≤20	2	13.3	2	13.3	4	13.3
21-30	3	20.0	2	13.3	5	16.7
31-40	0	0.0	1	6.7	1	3.3
41-50	5	33.3	4	26.7	9	30.0
51-60	3	20.0	2	13.3	5	16.7
61-70	2	13.3	4	26.7	6	20.0
Total	15	100.0	15	100.0	30	100.0

Of these 30 patients, 15 patients of low-grade glioma and 15 patients of high-grade glioma were selected. Average age of low-grade glioma was found to be 37.62 years, whereas, the average age of high-grade glioma was 45 years.

TABLE 2. The age-wise distribution of glioma

Age	Low Grade		High grade		Total	
	N	%	N	%	N	%
<= 20	2	13.3	2	13.3	4	13.3
21-30	5	33.3	0	0.0	5	16.7
31-40	1	6.7	0	0.0	1	3.3
41-50	2	13.3	7	46.7	9	30.0
51-60	3	20.0	2	13.3	5	16.7
61-70	2	13.3	4	26.7	6	20.0
Total	15	100.0	15	100.0	30	100.0

Of these 30 patients included in the study, 15 were male and 15 were female. The male to female ratio was 1:1. Out of 15 male patients, 6 had a low-grade glioma, and 9 had high grade glioma. On the other hand, in female patients, 9 had a low-grade glioma and 6 had a high-grade glioma.

TABLE 3. Gender-wise distribution of glioma

Sex	Low Grade		High grade		Total	
	N	%	N	%	N	%
Male	6	40.0	9	60.0	15	50.0
Female	9	60.0	6	40.0	15	50.0
Total	15	100.0	15	100.0	30	100.0

TABLE 4. Incidence of different types of low-grade glioma

Low Grade	n
Fibrilary Astrcytoma	1
Diffuse Astrocytoma	4
Gemistocytic Astrocytoma	4
Oligoastrocytoma	4
Pilocytic	2
Total	15

TABLE 5. Incidence of high-grade glioma

High grade	n
Anaplastic Astrocytoma	5
GBM IV	7
Oligoastrocytoma III	3
Total	15

Glioblastoma multiform GBM IV was found to be the most common pathological diagnosis among all patients (n=7).

Comparison of normalized area values of 3 metabolite resonances between low grade glioma and high-grade glioma is shown in Table 6. All 3 metabolites were found to be statistically significant (p<0.05).

TABLE 6. Pattern of metabolite resonances between low-grade glioma & high-grade glioma

	Low Grade Glioma (N=15)		High grade Glioma (N=15)		t	p
	mean	SD	mean	SD		
NAA	29.21	9.20	21.94	5.33	2.646	0.013
Choline (Cho)	82.98	7.44	92.20	3.77	-4.282	<0.001
Creatine (nCr)	45.20	4.91	31.64	5.34	7.248	<0.001

Graph shows 3 ROC curves of NAA, Cho and Cr for differentiation of low-grade glioma from high-grade glioma. Az value (under the ROC curve) is the highest in Cr and lowest in NAA.

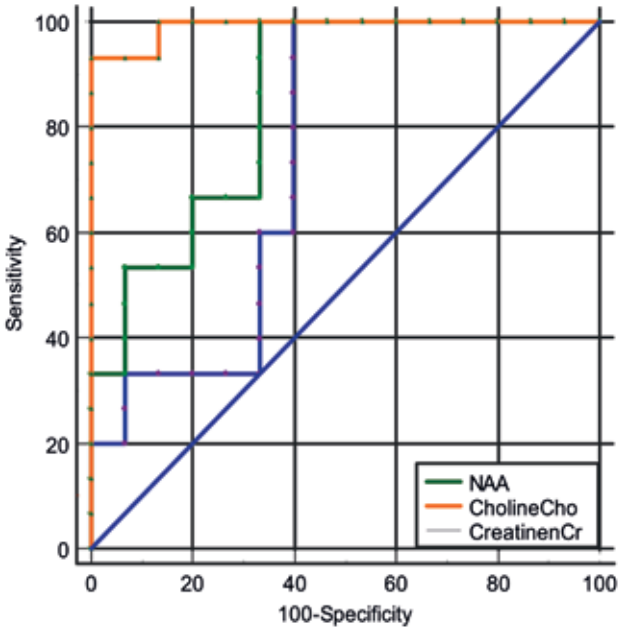

FIGURE 1. Graph shows ROC curves of Cho, NAA and Cr for differentiation of high-grade glioma from low-grade glioma

TABLE 7. Showing sensitivity and specificity of metabolite if differentiating low-grade and high-grade glioma

Metabolite	Sensitivity	Specificity
Cho	100	66.67
NAA	100	60
Cr	93.33	100

TABLE 8. Different metabolite ratios between low-grade glioma and high-grade glioma

	Low Grade Glioma (N=15)		High grade Glioma (N=15)		t	p
	mean	SD	Mean	SD		
Cho/Cr	1.84	0.13	2.88	0.70	-5.636	<0.001
Cho/NAA	3.13	1.10	4.46	1.15	-3.209	0.003
NAA/Cr	0.68	0.19	0.63	0.20	0.695	0.493

In our study, sensitivity and specificity of Cho/Cr in differentiating low grade and high-grade glioma are 93.33% and 100% respectively.

TABLE 9. Shows sensitivity and specificity of metabolite ratio in differentiating low-grade and high-grade glioma

Metabolite	Sensitivity	Specificity
Cho/Cr	93.33	100
Cho/NAA	100	66.67
NAA/Cr	100	46.67

Our study suggests that there a statistically significant difference is present among normalized area values of 3 metabolites (NAA, Cho, Cr) in differentiating low-grade from high-grade glioma. Significant difference is also present among Cho/NAA and Cho/Cr in differentiating low-grade from high-grade glioma. Sensitivity and specificity of Cho/NAA (sen-100%, spec-66.67%), Cho/Cr (sen-93.33%, spec-100%), and NAA/Cr (sen-100%, spec-46.67%).

DISCUSSION

We used our observations to make various comparisons with previous studies. In our study, 50% were male and 50% were females. In a study by R.K.A. Naser et al. in 2016, [26] 59.1% were males and 40.9% were females. In a study by Kim et al. in 2005, [27] 65.7% were males and 34.3% were females. Meanwhile, in a study by Q. Zeng et al. in 2011, 58.9% were males, and 41.1% were females.

TABLE 10. Comparison of glioma incidence in male and female with R.K.A. Naser et al., Kim et al., and Q. Zeng et al.

Sex	Present study	R.K.A. Naser et al. in 2016	Kim et al. in 2005	Q. Zeng et al. in 2011
Male	50%	59.1%	65.7%	58.9%
Female	50%	40.9%	34.3%	41.1%

According to the 2016 CNS WHO, the Glioma Grading for selected CNS tumors in our study was determined as low-grade (grades I & II), and high-grade (grades III & IV) for 50% patients in each group. In the study by R.K.A. Naser et al. in 2016, [26] glioma grading was determined at low-grade 41% and at high-grade in 59% of the patients respectively. In the study by Kim et al. in 2005, [27] glioma grading was determined at low grade in 20%, and at high grade in 80% of the patients respectively. In the study by Q. Zeng et al. in 2011, [28] glioma grading was determined at low grade in 33.3% and at high grade in 66.7% of the patients respectively.

TABLE 11

Glioma Grade	Present study	R.K.A. Naser et al., 2016	Kim et al., 2005	Q. Zeng et al., 2011
Low grade	50%	41%	20%	33.3%
High grade	50%	59%	80%	66.7%

In the study by Kim et al. in 2005, [26] different low-grade glioma versus high-grade glioma metabolite ratios for Cho/Cr were 2.17±0.79 (mean ± SD) and 1.52±0.66 (mean ± SD) respectively, with p<0.0001. In the study by Q. Zeng et al. in 2011, [28] different low-grade glioma versus high-grade glioma metabolite ratios for Cho/Cr were 2.94±1.83 (mean ± SD) and 1.72±0.62 (mean ± SD) respectively, with p<0.002. In the current study, the different low-grade glioma versus high-grade glioma metabolite ratios for Cho/Cr were 1.84 ± 0.13 (mean ± SD) and 2.88±0.70 (mean ± SD) respectively, with p<0.001.

TABLE 12. Comparison of different low-grade glioma versus high-grade glioma metabolite ratios for Cho/Cr with Kim et al., and Q. Zeng et al.

Metabolite Ratios Cho/Cr	Present study	Kim et al. in 2005	Q. Zeng et al. in 2011
Low-grade	1.84 ± 0.13	2.17 ± 0.79	2.94 ± 1.83
High-grade	2.88 ± 0.70	1.52 ± 0.66	1.72 ± 0.62
	(p<0.001)	(p<0.015)	(p<0.002)

The different low-grade glioma versus high-grade glioma metabolite ratios for Cho/NAA in the study by Kim et al. in 2005, [27] were 1.88 ± 0.96 (mean ± SD) and 2.02±0.94 (mean ± SD) respectively, with p>0.05. Meanwhile in the study by Q. Zeng et al. in 2011, [28] the different metabolite ratios were 1.97±1.10 (mean ± SD) and 3.65±3.14 (mean ± SD), with p < 0.05 for the same. In the current study, the different metabolite ratios were 3.13±1.10 (mean ± SD) and 4.46±1.15 (mean ± SD) respectively, with p<0.05.

TABLE 13. Comparison of different low-grade glioma versus high-grade glioma metabolite ratios for Cho/NAA with Kim et al. and Q. Zeng et al.

Metabolite Ratios Cho/NAA	Present study	Kim et al. in 2005	Q. Zeng et al. in 2011
Low-grade	3.13 ± 1.10	1.88 ± 0.96	1.97 ± 1.10
High-grade	4.46 ± 1.15	2.02 ± 0.94	3.65 ± 3.14
	(p<0.05)	(p>0.05)	(p<0.05)

At the same time, the different metabolite ratios between low-grade glioma and high-grade glioma for NAA/Cr in the study by Q. Zeng et al. in 2011, [28] were 0.89 ± 0.50 (mean ± SD) and 0.72 ± 0.35 (mean ± SD) respectively, with p<0.05. In the current study, the metabolite ratios were 0.68 ± 0.19 (mean ± SD) and 0.63 ± 0.20 (mean ± SD) respectively, with p>0.05.

TABLE 14. Comparison between different low-grade glioma versus high-grade glioma metabolite ratios for NAA/Cr with Q. Zeng et al.

Metabolite Ratios NAA/Cr	Present study	Q. Zeng et al. in 2011
Low-grade	0.68 ± 0.19	0.89 ± 0.50
High-grade	0.63 ± 0.20	0.72 ± 0.35
	(p>0.05)	(p<0.05)

The sensitivity and specificity of Cho/Cr in differentiating low-grade glioma from high-grade gliomas in our current study were found to be 93.33% and 100%, respectively. In a study by R.K.A. Naser et al. in 2016, [26] these values were found to be 62% and 75%, respectively. Similarly, in a study by Kim et al. in 2005, [27] the same were found to be 96.4% and 42.9%, respectively. In the study by Q. Zeng et al. in 2011, [28] the sensitivity and specificity were 84% and 83.33%, respectively.

TABLE 15. Comparison of sensitivity and specificity of Cho/Cr in differentiating low-grade glioma from high-grade glioma with R.K.A. Naser et al., Kim et al., & Q. Zeng et al.

Cho/Cr	Present study	R.K.A. Naser et al. in 2016	Kim et al. in 2005	Q. Zeng et al. in 2011
Sensitivity	93.33%	62%	96.4%	84%
Specificity	100%	75%	42.9%	83.3%

The sensitivity and specificity of Cho/NAA in differentiating low-grade and high-grade gliomas in the current study were found to be 100% and 66.67%, respectively. In a study by R.K.A. Naser et al. in 2016, [26] these values were found to be 72% and 90.9%, respectively. Similarly, in the study by Q. Zeng et al. in 2011, [28] the sensitivity and specificity were 88% and 66%, respectively.

TABLE 16. Comparison of sensitivity and specificity of Cho/NAA in differentiating low-grade glioma and high-grade glioma WHO with R.K.A. Naser et al. & Q. Zeng et al.

Cho/NAA	Present study	R.K.A. Naser et al. in 2016	Q. Zeng et al. in 2011
Sensitivity	100%	72%	88%
Specificity	66.67%	90%	66%

The sensitivity and specificity of NAA/Cr in differentiating low-grade glioma from high-grade gliomas in the current study were found to be 93.33% and 1007%, respectively. In the study by Q. Zeng et al. in 2011, [28] the sensitivity and specificity were 84% and 83.3%, respectively.

TABLE 17. Comparison of sensitivity and specificity of NAA/Cr in differentiating low-grade glioma from high-grade glioma WHO with Q. Zeng et al.

NAA/Cr	Present study	Q. Zeng et al. in 2011
Sensitivity	93.33%	84%
Specificity	100%	83.3%

In our study, the metabolite ratio of Cho/Cr and Cho/NAA were found to be statistically significant in differentiating low-grade glioma from high-grade glioma (p<0.05). Similar findings were presented in the study by Q. Zeng et al. in 2011.

CONCLUSION

Gliomas are the most common group of intracranial neoplasms. Though the gold standard technique for confirming diagnosis and classification is the histopathological examination, magnetic resonance spectroscopy (MRS) is increasingly reliable in differentiating between low grade and high-grade gliomas. This study has shown that Cho/NAA and Cho/Cr ratios are reliable in determining the tumor grade with good sensitivity and specificity. Thus, Cho/NAA

and Cho/Cr ratios of MRS maybe used as a diagnostic tool in differentiating low grade gliomas from high grade gliomas.

ETHICAL CONSIDERATIONS

Institutional ethical committee clearance was obtained and informed consent was taken from the participants. Confidentiality was maintained throughout the duration of study. Hard copies of the consent forms were kept with the primary researcher and will be destroyed 3 years after the completion of study.

Conflict of interest: none declared
Financial support: none declared

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