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" *Prediction of Glioma Grade by Radiological Analysis of its Metabolites* "

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ABSTRACT:

BACKGROUND: This study targeted to evaluate the ability of magnetic resonance spectroscopy (MRS) as a noninvasive predictive tool for histo-pathological grade of cerebral gliomas.

Results: Our study showed that 42/45 of the high grade glioma group (HGGG) had acquired markedly elevated Choline (Cho)/ Creatine (Cr) levels and 40/45 of them had acquired markedly elevated Choline (Cho)/ N acetyl aspartate (NAA) levels. On The other hand and across low grade glom group (LGGG); 13/15 lesions had acquired mildly elevated Cho/Cr levels and 14/15 lesions had acquired mildly elevated Cho/NAA levels with proved positive significant statistical correlation between Cho/Cr and Cho/NAA within the two groups and the detected histopathological data. Markedly elevated Cho/Cr and Cho/NAA levels were significantly correlated with HGGG; namely (grades III and IV). Mildly elevated Cho/Cr and Cho/NAA levels were correlated with LGGG; namely (grades I and II) ($p \leq 0.05$).

Conclusion: MRS can be used a predictor of glioma grade. The estimated MRS cut off values to prognosticate high grade glioma is ≥ 4.2 for Cho/Cr and ≥ 4.5 for Cho/NAA based on Receiver operating characteristic (ROC) analysis.

Key words: MRS - High grade cerebral gliomas - Low grade cerebral gliomas.

Introduction:

Gliomas are assorted histopathologically into four histopathological classes which help to regulate therapeutic designation and foretell consequences. [1] Low grade gliomas have low spread level, weak repetition capacity and favorable consequences. On the other hand; high grade gliomas tend to propagate, grow, recur and they carry poor outcomes.[2]

MRS is radiological modality using the magnet in calibrating tissue's metabolites; the most workable are (NAA), (Cho), (Cr), lactate and lipid.[3] Depiction of these tissue's metabolites aids in diagnosis of malignant processes and can distinguish regions of active malignancy from healthy brain tissue.[4]

Augmented (Cho) levels can be deemed as signal to cellular membrane turn-over. Decreased level of (NAA) refers to neuronal violation. Elevated (Cho/ Cr) ratio and (Cho/ NAA) ratio refers to malignant cellular membrane break-down and neuronal violation. Lactate doublets refer to non-aerobic metabolic vigor. Lipid peaks refer to necrotic alterations[5]; hence these alterations of metabolite ratios pointed to the class of glioma, also presence of lipid peaks and lactate inverted doublets points to aggressive gliomas.[6-7]

Materials and methods:

The current study was undertaken in Al-Nasr and Al-Salam hospitals, Port-Said governorate, Egypt from the beginning of April 2024 to the end of November 2024; all patients were presented by intra axial cerebral masses on the basis of MRI examinations and will be assessed by MRS for characterization of lesion's metabolites, the final diagnosis will be based on histopathological assessment.

Inclusion criteria were: a) All patients must be presented by intra axial cerebral space occupying lesions. b) All patients must be histopathologically assessed after open surgical biopsies or stereotactic needle biopsies. Exclusion criteria: a) Ineligibility for MRI. b) Hemorrhagic lesions containing blood components hinder MRS examination by its susceptibility artifacts. c) Any patient with cerebral mass proved pathologically to be non-gliomatous lesion. d) Any patient with previously managed cerebral masses; either surgical or radiotherapeutic management.

Agreement for this study was obtained from Port Said Faculty of Medicine Research Ethics Committee, University of Port Said, Egypt; (ethics committee reference number (ERN) is: MED (3/3/2024) s.no (149) RAD_006). All proceedings were done in conformity with the Declaration of Helsinki regarding research involving human subjects [8]. Written approval was acquired from all patients. Eligible patients were subjected to:

- **Pre-imaging clinical assessment:**

Full general and neurological examination of each patient was done by neurosurgery consultant with 10 years' experience within the outpatient clinic room with identification of the patient's complaints and presented clinical manifestations.

- **MRI imaging protocol:**

MRI examinations were done using 1.5 Tesla MRI devices (General Electric (GE), Milwaukee, USA) with the using of the adequate repetition time (TR), time to echo (TE) and inversion time (TI); axial T1 weighted image (WI), axial, sagittal and coronal T2WI, axial FLAIR, diffusion weighted imaging (DWI) and Susceptibility Weighted Images (SWI) were acquisitied. Injection of 0.10 millimole of gadolinium diethylenetriamine penta-acetic acid (GAD-DTPA) per kilogram body weight was done for some cases with acquisition of post contrast sequences.

MRS examination using point resolved spectroscopy sequence (PRESS) with chemical shift. The region of interest (ROI) is guided for the solid areas within the examined mass with avertion of any bleeding, necrosis, cysts and calcific foci. The ratio of Cho/Cr and Cho/NAA was numbered.

Radiological evaluation:

The radiological evaluation was achieved by 3 separate expert neuro-radiologists at least 10 years' experience according to the following steps:

1- Detection of the mass lesion by conventional MRI sequences; all hemorrhagic lesions, ischemic insults, extra axial lesions and demyelinating foci as well as any non tumoral findings were excluded. Each lesion was measured in its antero-posterior, side to side and cranio-caudal dimensions with characterization of its mass effects and surrounding peri lesional edema.

2- MRS evaluation by directing the region of interest (ROI) within the examined intra axial mass, peri-tumoral lesion, surrounding edema as well as the contralateral normal region with focusing upon the solid component and avoiding the necrotic areas, cystic regions, hemorrhagic areas and calcifications; the ratio of Cho/Cr and Cho/NAA was evaluated numerically with recognition of lipid peaks and lactate inverted doublets.

- **Surgical biopsies of the lesions:**

All lesions must be biopsied by neurosurgery consultant with 10 years' experience using open surgical biopsies or stereotactic needle biopsies.

- **Final microscopic assessment:**

All cases were examined histopathologically; the specimens were fixed in 10% formalin and examined by operative frozen section and detailed evaluation in the pathology department. The samples were paraffin firmed, cut, and stained with hematoxylin and eosin (H&E). These tumors were classified according to the 2016 World Health Organization (WHO) classification of central nervous system tumors.

- **Statistical analysis:**

The **Shapiro-Wilk test** was utilized to establish the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation (SD), median and interquartile range (IQR). **Mann Whitney test** for abnormally distributed quantitative variables to compare between two studied groups. **Spearman coefficient test** to correlate between two distributed abnormally quantitative variables. **Receiver operating characteristic curve (ROC)** is done with the area below it expresses the diagnostic achievement. Area more than fifty percentages denotes acceptable achievement and area about one hundred percentages is the best achievement for the test. It also grants a comparison of achievement between two tests. The significance of the acquired results was arbitrated at the 5% level.

RESULTS:

- **Demographic data:**

The study was carried out on 60 patients presented by multiple clinical manifestations, intra axial space occupying lesions were detected by MRI examination and final histopathological confirmations proved that all lesions were gliomatous; these patients ranging in age from 21 to 93 years with mean age 56.83±19.80 years. Within the study group 36/60 cases (60%) were males and 24/60 (40%) cases were females. **Table (1).**

- **Clinical data:**

Multiple clinical presentations were documented and most of the patients were presented by more than one symptoms, but the most common one was motor deficits which was detected in 33/60 patients and sensory abnormalities which was detected in 28/60 patients, tingling sensations was detected in 18/60 patients and disturbed level of consciousness was detected in 7/60 patients. **Table (1).**

Table (1): Distribution of the studied cases according to clinical data at the time of initial presentation: (n=60)

Clinical date	No. of cases	%
Sex		
Male	36	60%
Female	24	40%
Age (years)		
20 - ≤ 29	6	10%
30 - ≤ 39	8	13.33%
40 - ≤ 49	8	13.33%
50 - ≤ 59	12	20%
60 - ≤ 69	8	13.33%
70 - ≤ 79	9	15%
80 - ≤ 89	8	13.33%
90 - ≤ 99	1	1.67%
Mean and SD	56.83+19.80 years	
Clinical Presentations (Some cases showed more than1 presentation)		
Motor deficits	33	55%
Sensory deficits	28	46.67%
tingling sensations	18	30%
Disturbed level of consciousness	7	11.67%

- **Imaging data**

All patients were assessed by conventional MRI; lesions on left side of the brain parenchyma were detected in 39 cases and on right side of the brain parenchyma were detected in 19 cases, multifocal glioma detected in 1 case and butterfly glioma involving both sides of the brain occurred in the remaining case. Some of the lesions were involving more than 1 cerebral lobes but the most commonly affected lobe throughout the study group was the temporal lobe which was involved in 19/60 cases followed by the frontal lobe which was involved in 18 cases and the parietal lobe which was involved in 17 cases, occipital lobe was involved in 6 cases and the cerebellum as well as the posterior fossa were involved in 7 cases. **Table (2)**

MRS findings were studied; the largest Cho/ Cr ratio was 14.7 and the lowest was 1.9 with mean 7.04$\pm$3.45, the largest Cho/NAA ratio was 15 and the lowest was 2.3 with mean 7.72$\pm$3.94, lipid peaks and lactate inverted doublets were detected in 46 cases. **Table (3)**

Table (2): Distribution of the studied cases according to conventional MRI findings: (n=60)

Conventional MRI findings	No. of cases	%
Affected side		
Left	39	65%
Right	19	31.67%
Multifocal glioma	1	1.67%
Butterfly glioma	1	1.67%
Affected lobe (Some cases showed more than one affected lobe)		
Frontal lobe	18	30%
Parietal lobe	17	28.33%
Temporal lobe	19	31.67%
Occipital lobe	6	10%
Cerebellum and posterior fossa	7	11.67%

Table (3): Distribution of the studied cases according to MRS findings: (n=60)

MRS findings	Ratio
Cho/Cr.	
Mean ± SD.	7.04 ± 3.45
Median (Min. – Max.)	6.85 (1.90 – 14.7)
Cho/NAA	
Mean ± SD.	7.72 ± 3.94
Median (Min. – Max.)	7.55 (2.30 – 15.0)

- **Histo-pathological assessment:**

Different histopathological glioma types were detected considering that grades I and II are low grade glioma classes, on the other hand; grades III and IV are considered high grade glioma classes. Most common glioma type throughout the study group was glioblastoma multiform (GBM) which was detected in 25/60 cases followed by anaplastic astrocytoma which were detected in 20/60 cases. **Table (4)**

Table (4): Distribution of the studied cases according to final histopathological diagnosis: (n=60)

Histopathological findings	No. of cases	%
Glioma type		
Ganglioglioma	1	1.67%
Subependymal giant cell astrocytoma	1	1.67%
Ependymoma	1	1.67%
Oligodendroglioma	3	5%
Low grade astrocytoma	9	15%
Anaplastic astrocytoma	20	33.33%
Glioblastoma multiform	25	41.67%
Glioma grade		
Grade I	2	3.33%
Grade II	13	21.67%
Grade III	20	33.33%
Grade IV	25	41.67%
Glioma class		
Low grade glioma	15	25%
High grade glioma	45	75%

Our study showed that 42/45 of the high grade gliomas had acquired markedly elevated Cho/Cr levels and 40/45 of them had acquired markedly elevated Cho/NAA levels. **(Figure 1)** On The other hand and across low grade glioms; 13/15 lesions had acquired mildly elevated Cho/Cr levels and 14/15 lesions had acquired mildly elevated Cho/NAA levels.**(Figure 2)** The mean Cho/Cr throughout high grade glioma group was 8.41 ± 2.84 and this is significantly higher than the corresponding ratio throughout low grade glioma group which was 2.91 ± 0.81 with proved positive significant statistical correlation between Cho/Cr within the two studied groups and the detected histopathological data. Also the mean Cho/NAA throughout high grade glioma group was 9.25 ± 3.32 and this is significantly higher than the corresponding ratio throughout low grade glioma group which was 3.11 ± 0.67 with proved positive significant statistical correlation between Cho/NAA within the two studied groups and the detected histopathological data. As markedly elevated Cho/Cr and Cho/NAA levels were significantly correlated with high glioma classes; namely (grade III and grade IV). Mildly elevated Cho/Cr and Cho/NAA levels were correlated with low glioma grades and classes; namely (grade I and grade II). ($p \leq 0.05$). **(Tables 5-6).**

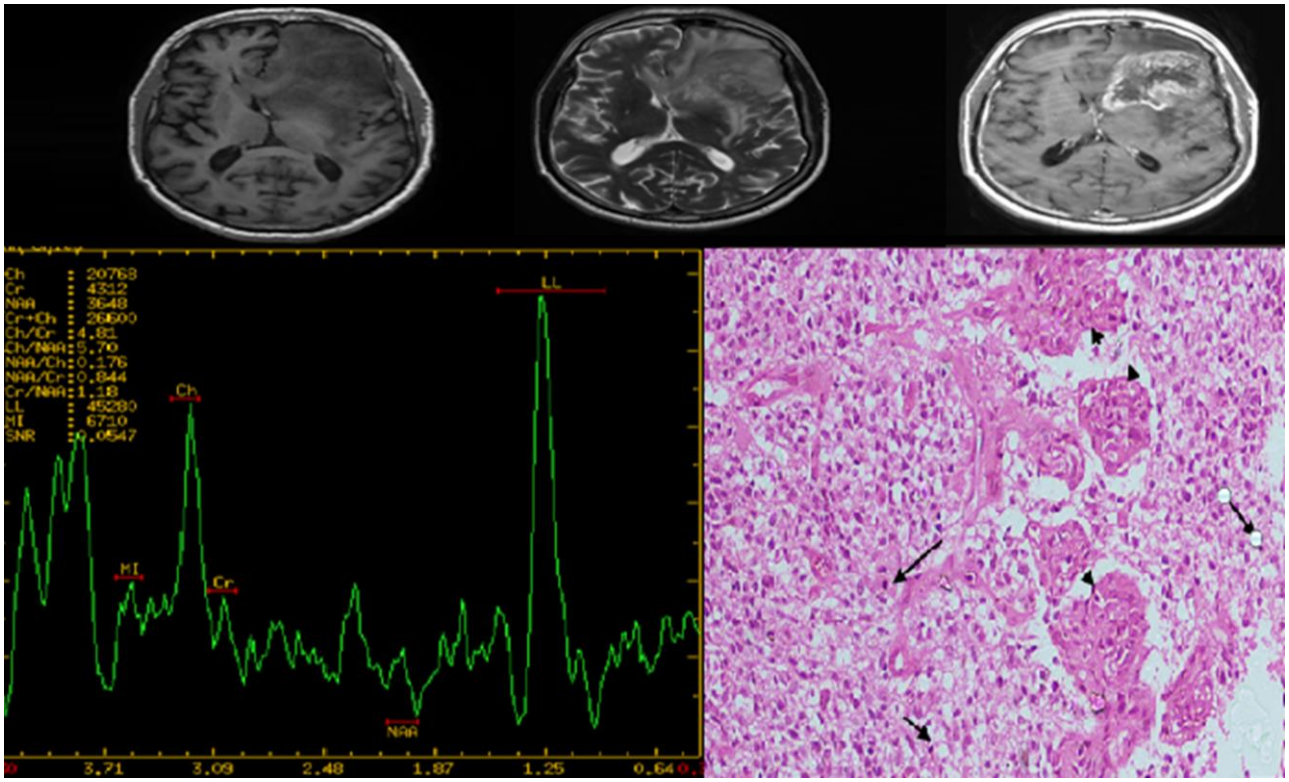

Figure (1) left frontal GBM: 72 years old male patient complaining of convulsions since 2 months. A) Axial T1WI B) Axial T2WI C) Post contrast axial T1WI show irregularly enhancing centrally necrotic left frontal mass D) MRS showing left frontal mass lesion with markedly elevated Cho/Cr = 4.81 and markedly elevated Cho/NAA = 5.70 E) Pathologically proved GBM with microscopic picture shows hypercellular tumor composed of atypical cells with hyperchromatic elongated nuclei, mitotic activity (arrows) & microvascular proliferation (arrows head) (H&Ex20).

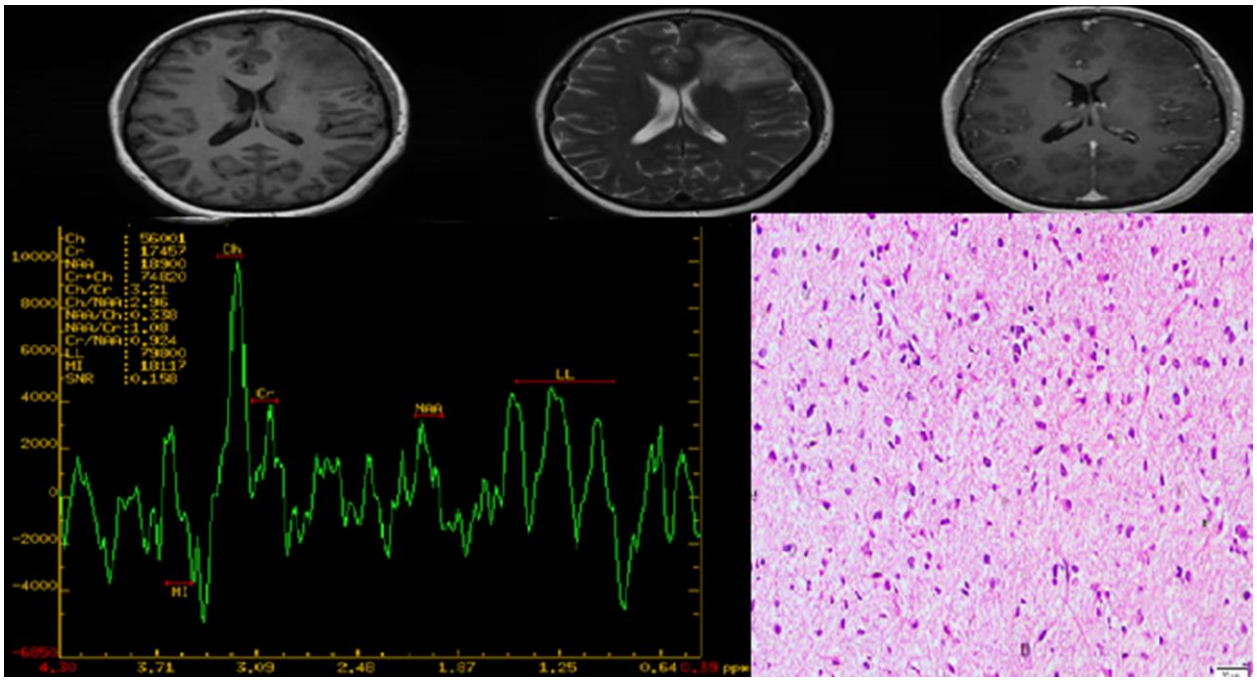

Figure (2) left frontal low grade astrocytoma: 48 years old female patient complaining of convulsions since 2 months. A) Axial T1WI B) Axial T2WI C) post contrast Axial T1WI show mildly enhancing left frontal mass D) MRS showing left frontal mass lesion with mildly elevated Cho/Cr = 3.21 and mildly elevated Cho/NAA = 2.96; this lesion was proved pathologically to be low grade astrocytoma. E) Microscopic picture shows hypercellular tumor composed of cells with elongated astrocytic nuclei and fine fibrillary processes on background with microcysts (H&Ex10)

Table (5): Relation between histopathology class and Cho/Cr. (n = 60)

	Histopathology classes		U	p
	Low grade glioma class (n = 15)	High grade glioma class (n = 45)		
Cho/Cr.				
Mean ± SD.	2.91 ± 0.81	8.41 ± 2.84	15.00*	<0.001*
Median (Min. – Max.)	2.60 (1.90 – 4.20)	7.80 (3.10 – 14.70)		
Cho/NAA				
Mean ± SD.	3.11 ± 0.67	9.25 ± 3.32	28.0*	<0.001*
Median (Min. – Max.)	2.80 (2.30 – 4.50)	9.80 (2.70 – 15.0)		

SD: **Standard deviation** **U**: **Mann Whitney test**
p: p value for comparing between Low and High grade gliomas
*: Statistically significant at p ≤ 0.05

Table (6): Correlation between histopathology grade and Cho/Cr. (n = 60)

	Histopathology grade	
	r_s	p
Cho/Cr.	0.742*	<0.001*
Cho/NAA	0.763*	<0.001*

r_s: Spearman coefficient
*: Statistically significant at p ≤ 0.05

The ROC curve for Cho/Cr in prognosticating glioma grade Figure (6) shows that the area below the curve is 0.978 with high statistical significance (p≤ 0.05) and its 95% confidence interval was (0.948 – 1.008). The sum of sensitivity and specificity is most achievable when using cut off value of Cho/Cr ≥4.2 i.e. sensitivity is 93.33%, and specificity is 100% while utilizing this cut off value. The achieved positive predictive value was 100%, the achieved negative predictive value was 83.3%. So; cut off value of Cho/Cr to suspect high grade glioma is ≥4.2, in other words; 93.33% of gliomas with Cho/Cr higher than or equals 4.2 would be high grade gliomas. **Figure (3) Table (7)**

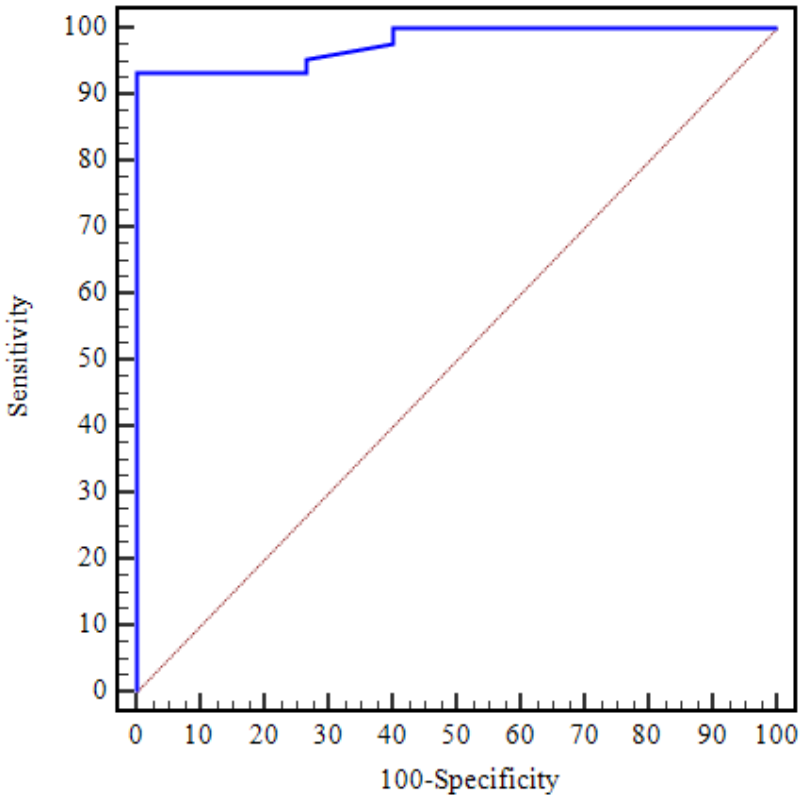

Figure (3): ROC curve for Cho/Cr. to discriminate patients with high grade gliomas (n = 45) from low grade gliomas (n = 15)

Table (7): Diagnostic achievement for Cho/Cr. to discriminate patients with high grade gliomas (n = 45) from low grade gliomas (n = 15)

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
Cho/Cr.	0.978*	<0.001*	0.948 – 1.008	**≥4.2#**	93.33	100.0	100.0	83.3

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at p ≤ 0.05
#Cut off was choose according to Youden index

The ROC curve for Cho/NAA in prognosticating glioma grade Figure (6) shows that the area below the curve is 0. 959 with high statistical significance (p≤ 0.05) and its 95% confidence interval was (0.912 – 1.005). The sum of sensitivity and specificity is most achievable when using cut off value of Cho/NAA ≥4.5 i.e. sensitivity is 88.89%, and specificity is 100% while utilizing this cut off value. The achieved positive predictive value was 100%, the achieved negative predictive value was 75%. So; cut off value of Cho/NAA to suspect high grade glioma is ≥4.5, in other words; 88.89% of gliomas with Cho/CAA higher than or equals 4.5 would be high grade gliomas. **Figure (4) Table (8)**

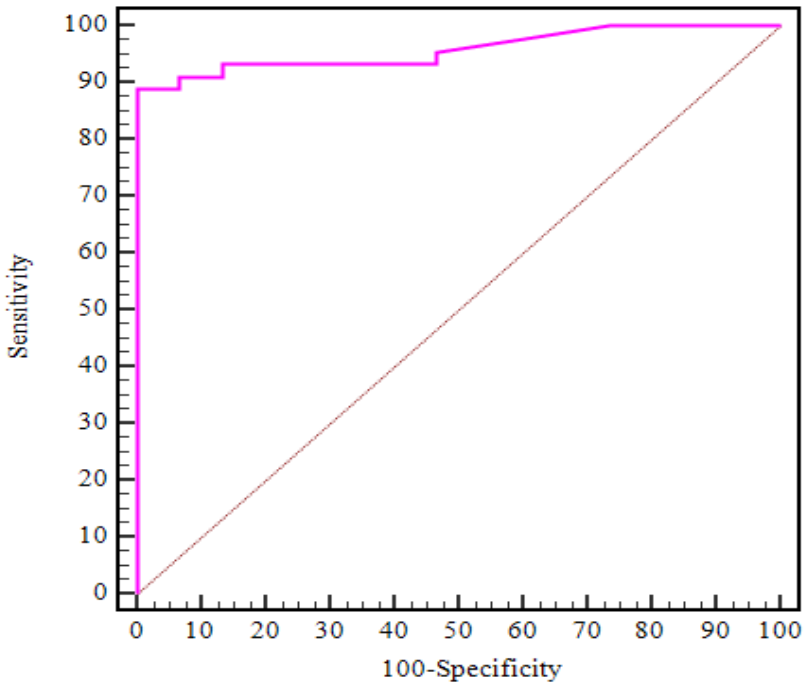

Figure (4): **ROC curve for Cho/NAA to discriminate patients with high grade gliomas (n = 45) from low grade gliomas (n = 15)**

Table (8): **Diagnostic achievement for Cho/NAA to discriminate patients with high grade gliomas (n = 45) from low grade gliomas (n = 15)**

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
Cho/NAA	0.959*	<0.001*	0.912 – 1.005	**≥4.5#**	88.89	100.0	100.0	75.0

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at p ≤ 0.05
#Cut off was choose according to Youden index

DISCUSSION

The ratio of the detected MRS metabolites within the cerebral glioma can prognosticate its grade and hence the prognostic criteria of the lesion and its behavior; even before the surgery and histopathological examination.[9] Our research aimed to assess the ability of MRS in prediction glioma grade. The question in our study was *Can the markedly elevated MRS metabolites within the cerebral glioma prognosticating that the glioma grade is high?* Our study included 60 patients ranging in age from 21 to 93 years with mean age 56.83+19.80 years; but the study of Rafique Z, etal included 80 patients with lower age group ranging from 13 to 80 years with mean age of 49.5 years and this is explained by focusing of our study upon higher group population. Within the study group 45/60 cases (75%) were high grade gliomas and 15/60 (25%) cases were low grade gliomas; this is near similar to the percentage of the study of Rafique Z, etal which contained 23.75% of patients had low-grade tumors, while 76.25% had high-grade tumors. [10]

Our study proved positive significant statistical correlation between Cho/Cr and Cho/NAA values with the detected histopathological data and this is matching with the study of Law M, etal.[11] It proved that markedly elevated Cho/Cr and Cho/NAA levels were significantly correlated with high glioma classes. Mildly elevated Cho/Cr and Cho/NAA levels were correlated with low glioma grades and classes; this is similar to the results of Yerli H, etal. [12]

Using ROC analysis throughout our study proved that the Cho/Cr cut off value to prognosticate high grade glioma is ≥ 4.2 and this value is near the value of Shakir TM, etal who stated that the Cho/Cr ratio for differentiating glioma grade was 3.72, also our study proved that Cho/NAA cut off value to prognosticate high grade glioma is ≥ 4.5; but Shakir TM, etal stated that Cho/NAA value for differentiating glioma grade was relatively lower and measuring 3.14; this is can be explained by the large dominant proportion of high grade glioma lesions throughout our study which was 45/60 (75%) of the total cases and this is elevating the level of Cho/Cr and Cho/NAA cut off values.[13]

Points of strength: The study was done using 1.5T MRI device with multidisciplinary team of neurosurgeon, histopathologist and 3 radiologists.

Limitations of the study: The study didn't include pediatric age group due to limited pediatric presentation within the study group.

CONCLUSIONS:

MRS can be used for prediction of glioma grade as a radiological non-invasive tool. Positive significant statistical correlation was proved between elevated Cho/Cr and Cho/NAA values with high gloma grades. Cut off value of Cho/Cr is ≥4.2 and of Cho/NAA is ≥4.5 to predict high grade glioma.

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