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Magnetic resonance spectroscopic metabolites as prognostic factors for survival and recurrence compared to anatomic MRI in grade III gliomas post adjuvant radiation: A retrospective analysis

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Abstract

Objectives: *The primary objective was to assess the prognostic significance of MR spectroscopy parameters (choline/NAA and choline/creatine ratio)both at Baseline (Pre operative MRS) and post adjuvant radiation regarding survival and its comparison with traditional anatomic MRI based grading parameters (enhancement quality, enhancement proportion, enhancement margin,T1 Flair Ratio).A secondary objective was to corelate any association between the MRS metabolic baseline and response values(cho/NAA and cho/creatine) with Histopathologically proved recurrence.*

Materials and Methods: *25 histopathologically proved Grade III gliomas (astrocytomas and mixed oligoastrocytomas),registered in our institution between 2013-2016 who had both preoperative and post radiation MRI and MRS done were included in the study.MRS metabolic parameters were graded at baseline (Pre op) into cho/Naa ratio (>3.5,<3.5)and cho/creatine ratio(>2,<2) and at response(post adjuvant radiation) into cho/Naa change(>25%,<25% of baseline) and Cho/creatine (>10%,<10% of baseline). Baseline Anatomic MRI characteristics of the tumor (pre op) was also graded into enhancement quality (mild/avid), proportional enhancement(>50%,<50%), Margin of enhancement(well defined/poorly defined) and T1/Flair Size ratio(expansive/infiltrative)based on the VASARI/REMBRANDT MR feature set. PFS was estimated from time of completion of adjuvant treatment to clinical or radiological progression or last clinical follow up. Univariate analysis using Kaplan maier survival method and Log Rank value test was done for both the metabolic MRS value groups (baseline and Response) and the anatomic MRI parameters. Univariate survival analysis was also done to assess significance of Radiation dose (>50 Gy, <50 Gy) and extent of surgery (total vs subtotal/biopsy). Any parameter with log rank p value<0.08 was deemed to be significant and was entered into multivariate cox regression analysis. Histopathologically confirmed cases of recurrence (positive HPE/negative HPE) was correlated with baseline and response MRS value groups using Paired t test to corelate any significance. All analysis were done using SPSS-V23.*

Results: *Median follow up period was 38 months &median pfs was 13.2 months. On univariate analysis of the baseline and response value groups of MRS, the most significant factor associated with better survival*

was cho/cr change greater than 10%(PFS of 22.1 months vs 9.3 months, log rank p value=0.002) followed by baseline cho/naa less than 3.5 (PFS of 21.7 months vs 12.7 months, p=0.021).Among the Anatomic MRI parameters well defined enhancement margin was associated with survival advantage(PFS 20.5 vs 12.3 months, p=0.045). Full RT dose and total excision both were individually associated with better survival (p=0.073 and p=0.002).On multivariate analysis only cho/cr change >10% was significant at p=0.144 among MRS parameters. Among the 7 patient who underwent rexcision following clinical/radiological progression,6 were HPE confirmed recurrence. On paired t test Cho/naa change>25% of baseline(post RT response value) was identified as the best predictor of HPE confirmed recurrence (p=0.008) better than radiological progression during Follow up(p=0.350)

Conclusion: *MRS metabolic parameters (Lower baseline cho/naa and greater cho/cr change after treatment) are of significant survival advantage whereas lesser cho/Naa response has better specificity for HPE proved recurrence than its anatomic counterpart. Prospective studies evaluating voxel based MRS data incorporated into treatment planning systems can be an interesting way forward.*

Keywords: *Magnetic resonance spectroscopy, High grade gliomas, Adjuvant radiation, recurrence, progression free survival, radiotherapy planning.*

Introduction

Prognosis of glial tumors is inherently complex with a significant number of factors contributing in an asymmetric and multidimensional way. The inclusion of genetic mutations in the WHO 2016 CNS tumor classification[1] has paved the roadway for newer criteria of risk stratification. However Response assessment with Traditional anatomical MRI imaging has its shortfall with the inability to measure the physiological and metabolic response of the Tumor to treatment. Although Sensitivity is equivalent or non-superior to, Specificity criteria in determining recurrence in response assessment studies which included Physiological and metabolic parameters like Diffusion weighted MRI[2] and MR Spectroscopy[3] are consistently higher.

Response assessment in treated Grade II and Grade III Glioma most commonly is done using a combination of clinical symptoms and Anatomical MRI based evidence of progression[4]and is not commonly adjuncted with functional, physiological, and metabolic parameters as is commonly the case with GBM[5].With the advent and ease of generating both structural features and metabolic functions in the same setting ,much more deeper insight into the individual tumor behaviour post treatment can be generated[6] with adjuction of spectroscopy to traditional anatomic MRI. The fusion of MRS metabolic data into primary treatment planning also showed increased total target volume with subsequent site modification to the boost volume, both of which was Predicted to be associated with Improved local control[7] Physiological and Metabolic character of the tumor in the pre treatment stage and their graded response post treatment is itself a recognised independent prognostic Factor[8] and their incorporation and correlation with other patient, tumor and treatment related factors have the potential to identify the cohort at risk for early recurrence. Voxel based progression studies have shown that higher choline NAA index voxels in pretreatment planning volumes have higher chance of recurrence[9] with moderate influence of dose escalation at those sites

In our present study we have aimed to study the significance of Metabolic MR parameters (both at baseline and response level) as outcome predictor in Grade III Gliomas who received post operative adjuvant radiotherapy. Other objectives included comparison of strength of positive predictive association between Pre op anatomic MRI tumor features (as per REMBRANDNT) [10]to that of Baseline MRS values regarding progression free survival. Positive Predictive value of HPE proven recurrence was also comparatively correlated between radiological anatomic MRI based progression and Response value groups of MRS.

Materials and Methods

Inclusion Criteria: Retrospectively patient data was collected and the patient who fulfilled the following criteria was included in the study

- Preoperative T1 and T2 weighted MRI
- Preoperative MR spectroscopy (single voxel/ Multivoxel)
- Received at least 50 Gy external beam radiation
- Post adjuvant radiation MRS
- KPS >70 at the start of Radiation
- Astrocytoma or Mixed astrocytoma variant

Treatment Pattern

25 patients (table 1) Grade III Gliomas were enrolled in our study who had underwent either total/Subtotal excision or a biopsy and had a Pre op MRI and MRS (table 2) and were treated with adjuvant radiation. External beam radiation in our institution was done with Philips CT simulation wth slices of 3mm and then planning was done in Varian Eclipse Treatment planning system(v 13.1).A portion of the study population also received Adjuvant radiation in outside centres. In all 8 patients were treated with conformal radiation(3D CRT and IMRT),12 with CT simulation and volumetric treatment planning but treatment delivery in cobalt without Multi leaf colimeter and 5 were treated with 2D X-ray based simulation. Patients were treated with doses of 50-54 Gy in standard fractionation. Some Patients were treated with a hypofractionated schedule in phase I followed by phase II bringing the total dose to 50-54 Gy in EQD2.All patients received at least 50 Gy External beam radiation.13 patients received concurrent Temozolamide at 75 mg/m2/daily and only 3 patients, maintanence doses at 150-200 mg/m2 D1-5 in a 28 days cycle with an average of 4 cycles with the maximum of 6 cycle for 1.

MRS Metabolic parameters

Baseline (Pre OP) MR metabolic values of Choline/ Naa (ratio) were grouped as >3.5 and<3.5 for with the cutoff for the groups being the approximate median value of the baseline cho/naa ratio in the total patient population. Baseline values of Cho/cr(ratio) were also stratified into 2 groups with values>2 and <2 based on the approximate median value seen in the cohort . Response data from the post treatment MRS (the last one done)was calculated and grouped as decrease in cho/naa (ratio) >25% or<25% from baseline value based on previous literature data suggesting the subgroup with increased change has better DFS[11] median change in both the groups. Cho/cr (ratio) response from baseline also was grouped into >10% or<10% based on the median change from baseline in the population cohort

Anatomic MRI Parameters

4 Pre op anatomic MRI based tumor specific features based on the VASARI MR scale namely contrast enhancement quality (mild/avid), Proportional enhancement (>50%,<50%), Contrast margin (well defined/poorly defined) and T1/FLAIR size ratio (Infiltrative/Expansive) (if FLAIR was not available T2 sequence was used)

End Points

- From completion of adjuvant treatment to clinical or radiological progression (progression free survival analysis)
- From completion of adjuvant treatment to detection of Histopathological recurrence (recurrence analysis)

Survival Analysis

Univariate survival analysis was carried out using Kaplan Maier analysis And Log rank test with respect to both the baseline MR metabolic value groups and the metabolic response value groups Anatomic MRI parameters (Pre op baseline values) were also analysed by univariate survival analysis (Kaplan Maier) and log rank test

All factors significant at P<0.08(log rank) in univariate analysis was entered in a Multivariate cox regression analysis (Forward LR)

Paired t test was also used to compare the specificity of Baseline and response metabolic value groups with anatomic MRI based progression in the diagnosis of HPE proven recurrence. A p value less than 0.08 is taken to be of significance

All calculations were done in SPSS (v-23.0)

Table 1 Baseline Patient and tumor characteristics

Patient characteristics (n=25)		High grade gliomas (n=25) Mean(n)	Percentage
Age	<40yr	27.8 yrs	63.3
	>40yr		37.7
Tumor size	>3.5 cm	4.2cm	66.7
	<3.5cm		23.3
Hemisphere	Right		65.4
			34.6
Contrast enhancement quality (T1 post contrast)	minimal		60
	avid		40
Proportional contrast enhancement (T1 post contrast)	>50%		40
	<50%		60
Contrast Margin	Well defined		52
	Poorly Defined		48
T1/FLAIR (tumor size ratio)	Infiltrative		44
	Expansive		56
Extent of surgery	Total excision		48
	Subtotal + Biopsy		52
Response assesment	CR		6.2
	PR		93.8
Histology	Astrocytoma		86.7
	Mixed oligoastrocytoma		13.3

Table 2 MRS metabolite Ratios (baseline and response)

MRS metabolic values at baseline		Grade III gliomas(n=25) Median	Range	percentage
Cho/Naa Level	>3.5	3.6	2.1-5.3	56
	<3.5			44
Cho/Cr level	>2	1.98	1.10-3.10	52
	<2			48

MRS metabolic values(response) In terms of percentage change of baseline value		Grade III gliomas (n=25) Mean change (percentage)	Percentage
Cho/naa	>25%	30.5	54
	<25%		46
Cho/cr	>10%	11.1	50
	<10%		50

Results

Median pfs for the total cohort was 13.2 months. Univariate analysis using Kaplan maier survival model for baseline MRS metabolic values showed significant association with cho/naa >3.5 (Pfs of 21.7 months vs 12.7 months, p=0.021)(table 4). Univariate analysis using Kaplan maier survival model for MRS metabolic response value groups showed significant association of cho/cr change greater than 10% of baseline (PFS of 22.1 months vs 9.3 months, P=0.002) (table 4)

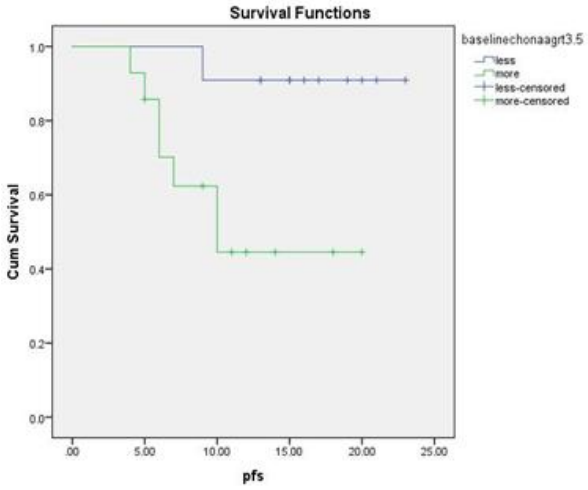
Table 4 Univariate survival analysis of baseline and response MRS value groups

Baseline MRs metabolic value groups		Grade III gliomas (n=25) PFS	P value
Cho/naa	>3.5	21.7	0.021
	<3.5	12.7	
Cho/cr	>2	19	0.46
	<2	14.6	

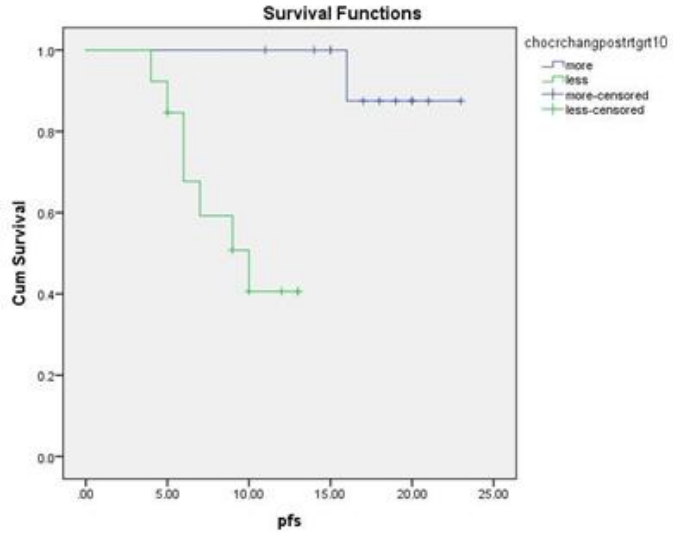
Univariate survival analysis of anatomic MRI based tumor characters (based on VASARI criteria) showed most significant association with the enhancement margin feature (well defined vs poorly defined) with PFS (20.5 months vs 12.3 months, log rank p value=0.045) and the quality of enhancement character (mild vs avid) was assessed to be moderately associated with survival (PFS 19.2 vs 12.6 months, log rank p value=0.185) (Table 5)

Table 5 Univariate survival analysis of baseline Anatomic MRI parameters

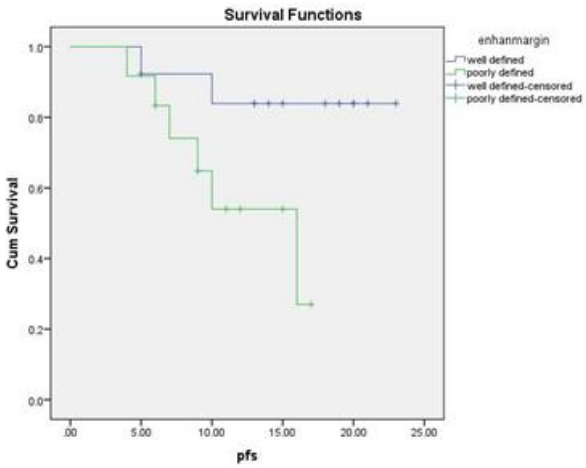
Anatomic MRI characters of tumor		Grade III gliomas (n=25)	
		PFS	P value
Enhancement quality	mild	19.5	0.185
	avid	12.6	
Proportional enhancement	>50%	17.8	0.925
	<50%	15.6	
Margin of enhancement	Well defined	20.5	0.045
	Poorly defined	12.3	
T1/Flair size	Infiltrative	18.7	0.375
	Expansive	15.2	



Kaplan maier showing significant association with baseline cho/naa>3.5 (Pfs of 21.7 months vs 12.7 months, p=0.021)



Kaplan maier showing significant association of cho/cr change greater than 10% of baseline (PFS of 22.1 months vs 9.3 months, P=0.002)



Kaplan maier showing significant association with the enhancement margin feature (well defined vs poorly defined) with PFS (20.5 months vs 12.3 months, log rank p value=0.045)

Surgical extent (Total vs Subtotal/Biopsy) was identified as a very significant factor for survival on univariate analysis (PFS of 22.1 vs 10.1 months, Log rank p value=0.002) On entering the above factors in cox regression Multivariate analysis, Cho/cr Change greater than 10% of baseline retained significance at somewhat moderate level (P=0.144) only next to surgical extent(P=0.023).

Out of the 25 patients that received adjuvant radiation 7 patients had resurgery in the post progression follow up period with HPE proven recurrence in 6 of them. Cho/Naa change <25% was positively and strongly associated with biopsy proven recurrence (P value=0.008) (paired t test) compared to Radiological progression during follow up (p value=.350) (table 9). Receiving radiation dosage of <50 Gy was also correlated positively with biopsy proven recurrence (p=.088). Metabolic Respone in the Cho/cr value group was not significantly associated with HPE proven recurrence ((p=0.350) and no baseline MRS metabolic value groups were correlated with biopsy proven recurrence (Table-6)

Table 6-Paired t-test correlating MRS metabolic parameters and anatomic radiological progression with HPE proven recurrence

HPE proven recurrence with metabolic parameters		Hpe proven recurrence		P value
		yes	no	
Cho/Naa change of baseline	>25%	0	1	P=0.008
	<25%	6	0	
Anatomical MRI based high grade Progression	Yes	3	0	P=0.350
	No	3	1	

Discussion

Metabolites quantifiable in a clinical MRS with a proven role in prognostic significance are choline(cho), N-acetylaspartate (NAA) and creatine (Cr)[12]. Whereas observer viewed different parameters of structural orientation of the tumor like midline shift, tumor diameter and patient and tumor factors like diameter, midline shift and objective patient and tumor related factors were both identified as of prognostic significance in Low grade Glioma[13] the anatomic features were not of that significant value in Grade III gliomas[14].This literally paved the idea for increased focus on linear & individualisation of tumor characterisation with higher grades of Glioma, as in GBM we have an established significance for Functional imaging be it DOPA PET[15]or MR spectroscopy[16].

In our current study we retrospectively identified Patients of WHO grade III gliomas of astrocytoma variant, who underwent post operative radiation, either in our institution or other with techniques ranging from 2d x ray based simulation to IMRT, however within this wide spread range of Radiation Techniques ,in our inclusion criteria we maintained a homogenous intake which was, availability of at least 1 Pre operative MRI (at least T1 and T2 sequences) & MRS and 1 MRS post adjuvant radiation (92% was within 6 months of finishing adjuvant radiation)(Both multivoxel/CSI or single voxel) but about 90% of our MRS data both Pre operative and Adjuvant radiation were based on Multivoxel data, probably signifying the in homogeneity of metabolites that Grade III gliomas produce due to their increased infiltrative pattern[17]. Also regarding the use of absolute concentrations vis a vis the ratios in terms of both initial grading and in prognosis[18], although normalized value of Creatine has been established as a prognostic factor at baseline[19] ,in other studies regarding increased survival after adjuvant radiation, cho/cr response pattern were deemed to be of significance[20]. Infact Quon *et al*, showed that >40% change in normalized choline from week 4 to 2 months post RT was associated with both poor OS and PFS. In our study we could not provide both normalized and metabolite ratios in terms of significance due to lack of standardized MRS reporting system in our cohort. As patients were recruited retrospectively based on availability of pre op and post Radiation MRS data, standardization and uniformity regarding the radiotherapy techniques couldn't be ascertained, and this might have a influence on the response patterns[21]. In our study population 8 patients underwent conformal radiation (3DCRT and IMRT), 12 patients were treated with CT simulation and volumetric Treatment planning with treatment being delivered in cobalt machine without Multi leaf colimeter and 5 patients were treated with conventional 2D xray simulation.

The assessment of Cho/cr ratio response value Post adjuvant radiation as a significant prognostic factor for increased progression free survival in our study goes well with the established literature[22].

Increased Baseline Cho/NAA shown in our study linked to poor survival, which has been reported as of significant prognosis in newly diagnosed GBM[23],this has not been labelled of significance in Grade III gliomas until now. Cho/NAA index at the time of diagnosis is highest in grade III gliomas[24], owing to increased choline concentration due to increased cell membrane densities(compared to Grade II gliomas) and a concurrent decrease in NAA concentration due to the increased infiltrative character(again compared to Grade II).In Grade 4 (GBM), as most of the tumor mass is formed of the necrotic volume, the metabolite index values are generally low[25]. This hypothesis of tumor physiology is probably reflected in our recurrence

data too, where decrease in Cho/Naa index less than 25 % of baseline post adjuvant radiation, shows significant association with post rexcision HPE proven recurrence, compared to radiological progression. Traber *et all* showed that focal choline accumulation early after adjuvant radiation was umambigously predicting recurrence in High grade gliomas. Change in Cho/NAA index in Grade III gliomas, among all other grades, probably reflect the percentage reduction of functional tumor core, in the most significant manner, owing to the highest metabollicaly active to necrotic volume ratio. As Recurrence in Grade III gliomas in mostly within the Treated field[26],our study iterates the fact that prospective studies incorporating, the voxel based functional imaging data incorporated into the treatment volumes, might bring about better local control. Pirzkall *et al* (2001) showed that incorporation of MRSI data into anatomic MRI based radiotherapy treatment volumes alter both the primary treatment and the boost volume which was relatively validated in a study by Ballengrud (2011). Voxel based progression studies have shown choline NAA index (CNI) having the greatest association with recurrence, better than DWI parameters like ADC[27]. Zhang *et all* have shown incorporation of 3D-MRSI data have improved the primary surgical extent delineation in glioma surgery However till date no prospective study has shown survival advantage in HCG incorporating MRSI data in treatment volume.

As data was collected retrospectively In our study we couldn't estimate the role of surgery in the extent of response in MRS metabolite index, as most of the MRS were probably done during either routine follow up, or or clinical and radiological progression post adjuvant radiation, however as suggested post surgical MRS signal is most probably due to residual tumor margin, as opposed to post surgical change[28].

The overall prognostic significance parameters of MR spectroscopic metabolic index as compared to traditional anatomic MRI done in our Study is in concordance with established literature[29]. As a retrospective data push, it suggests incorporation of Functional parameters into treatment planning by means of software development. Although PET and planning CT fusion has been reported to improve GTV delineation, geographic misses, and a reduction in organ at risk doses[30], Integrated PET/CT simulation centres are few in number and of huge cost. CT-MR fusions have become a standard in treatment of Gliomas using linear accelerator, and incorporating MRS data taken at the same time as of diagnostic MRI seems to be of much cost effective method, if routine radiotherapy planning software starts incorporating them.

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