Evaluation of the role of metabolites in the diagnosis of the brain tumors using the MRS of the intensified nuclear magnet

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ABSTRACT

Nowadays brain tumors remain as a significant cause of morbidity and mortality and are often treatment refractory. The grading of brain tumor has an important implication in clinical management. Currently, magnetic resonance spectroscopy (MRS) is an important modality in evaluating and grading brain tumors. The aim of this study is evaluation of metabolites in the differentiation of brain tumors and grading of brain gliomas using HMRS (proton magnetic resonance spectroscopy).The studies were performed using single voxel MRS ،3Tesla with pulse of sequence used for MRS was point resolved single volume spectroscopy (PRESS)with repetition time (TR) 1000-6000ms and echo time (TE) 36-136ms and The detected distinguished metabolites was included Choline (Cho), Creatin (Cr), and N-acetyl a aspartate (NAA), 37cases had data which passed quality control. The Patient ages ranged from 7 To 81 years, 17 were male and 20 female.MRS data was processed using SYNGO software to give mean spectra and metabolite concentrations which were compared using min it a band SPSS. To test the primary hypothesis, univariate logistic regression was performed on each individual measured metabolite quantity. Significant differences were found in concentrations of key metabolites and Cho/NAA and Cho/Cr ratios using T-test and significance ($P \leq 0.05$). In the assessment of age rate and tumor grading, the malignancies of brain tumors did not correlate with patients' ages as well as sexuality. MRS can detect subtle differences between low grade brain tumors in children and should form part of the clinical assessment of these tumors.

Keywords: Spectroscopy; Brain Masses; Magnetic Resonance.

INTRODUCTION

Glioma is the most common type of primary brain tumors, and is grouped into four grades according to the WHO criteria, which Astrocyromas and Oligodendroglia's, Glioblastoma Multiforme (GBM) or grade IV astrocytoma are among the most common and lethal adult malignant glioma tumors [1,2]. Brain tumors are the top reasons of the death and the outbreak of diseases among all types of children's tumors and a new method is required improve the clinical management.Magnet Resonance Spectroscopy H^1 (MRS) allows the ingressive measurement of the ting molecules density in the tumor tissue in order to present the clinical useful radiological biomarkers[3]. Since the regular MRS and other biomedical radiological methods let us identify and locate the tumor only vaguely, in order to diagnose the tumors, biopsies are usually required. Unfortunately, a large number of tumors may not be accessible for biopsy; therefore, grading tumors using MRS has the additional advantage of an ingressive diagnosis method. MRS H^1 is a prominent method to evaluate the type and grade of tumor; it is also an important method to target and assess the reaction to a treatment [4]. MRS is increasingly used to evaluate the brain neoplasms. It indicates the biochemical and metabolic combinations of various types' brain tumors [5]. MRS not only can show the unusual finding in almost 100% of the brain tumors, but is also useful in distinguishing the tumors and specifying the metabolic changes in line with the tumor growth, the level of malignancy and the reaction to the treatment [6]. The MRS finding of the brain tumors,

demonstrate the increased Cho levels and the decreased level of the NAA compared to those in a normal brain which reflects the increased cellular tissues [7, 8]. Since tumors are heterogeneous with necrosis nucleus, aggressive developed margins from around the brain tissue, there seems to be a variety of spectra depending on the area which is sampled using MRS[9]. However, due to various toll, heterogenic, overlap among different type of tumors and the analyzing techniques, it is often difficult for physicians to merely use MRS for their diagnosis; therefore, MRS should be used as an auxiliary method and technique for diagnosis.

The purpose of this study is to evaluate the Choline (Cho),Cratine (Cr), N-Acetil-Aspartat metabolites and also dreading human brain tumors based on the metabolite ratio of Cho/Cr and Cho/NAA in an unaggressive way.The advance in radiology allows investing gate the characteristics of the tissue in an unaggressive way which offers mores more significant insights compared to the ones being offered by the tumor biology in in vivo[10]. Thus, the aim of modern radiology is not only to offer an unaggressive histological diagnosis but also to improve the categorization of the tumors. Using MRS-H starts with anatomical images; so that, in order to determine the volume (VOI), spectra are required. In order to provide the spectrum, different techniques including single- or multi-voxel spectroscopy using short and long time Echos (TE) might be used. Each technique has some pros and cons; therefore, choosing the right technique for a specific purpose is of great importance, because it will lead to improved quality of the resulted [11, 12].

MATERIALS AND METHODS

Regarding the conditions and facilitation, in the present study, evaluation of the patient's metabolites was conducted using single-Voxel Spectroscopy PERESS and an approximately long time of echo ranging from 36 to136 msec the replicating time ranging from 1000 to 6000 msec. Spectroscopy of the penitent's has been done using a 3T-MRI (3T Siemens Symphony Magnetron) which was single-Voxel (PRESS) and contained a set of square coils as its transmitter and receiver bonds. Experientially, the VOI of the MRS were inserted into the toll. Brain normal tissue, structures full of liquid and the fat tissue in the scalp were ignored. Water suppression, which is a part of pre-processing stages, was done prior to spectroscopy. The pulse sequence used for MRS was PRESS- point single-volume spectroscopy with 1000 to 6000 ms (TR) replicating time and 36 to 136 ms (TE) long-time echo. The voxel size was mostly 1.5*1.5*1.5 cm^3 for single-Voxel and in some case it was 2*2*2 cm^3. The matrix size was 16*16*1 and the scope was 160*160*15 mm^3. The obtained protocols for the clinical data were defined radiologically and his to pathologically in order to ensure the adaptability of the obtained data.

For Choline (in 3.22ppm), Cratine (3.02 to 3.04 ppm) and N-Acetil Aspartate (in 2.02 ppm) metabolites which are resonant in their characteristics, quantative analysis of the spectra were conducted. The peak data were recorded and used to calculate the Cho/NAA and Cho/Cr ratios. The resonance intensities of the metabolites were determined by fitting the peak points. When the resonance intensities of the metabolites reduced significantly and were not determined automatically, the peak points, using the graded scale of monitor or film. These valves were then put into Cho/NAA and Cho/Cr ratios.

The statistic calculations of the MRS data were first processed by SYNGO software in terms of mean spectra and the metabolite densities. They were, then, compared using Minitab and SPSS19 softwares. Diagrams were drawn using Excell. In order to test the main hypothesis, single-variable logistic regression was performed for each single measured value of metabolites.

Cho/ANN and Cho/were compared between tumor levels using parameter analysis. Metabolite ratios were also compared between glioma cases. Statistically, in a ≤ 0.05 significant mean deviations were observed.It is not needed to purchase an exclusive unit for this purpose to conduct MRS. As long as the scope his sufficient power, the majority of available MRS systems re scope-powers are able to be used. In the present study, 3 T scope- power has been used.

There is no additional preparation required for the patient. The patient has to remain immobile for around 4 minutes during sampling from every unit for single-Vexel spectroscopy. The general trend for the evaluation of the brain images as well as the

sampling from each unit takes about 30 minutes. Evaluating tumors based on the charges of the density of the common metabolites in almost-long including Cho, Cr, NAA and also Cho/NAA& Cho/Cr was done.

37 participants had the acceptable data for the quality control; 30 of them had brain tumors (20 females and 17 males with their age ranging from 7 to 81 years old and the mean age of 41.46± 2.78 years old) and 7 out of 37 were normal with their age ranging from 38 to 51 years old (m males and 4 females and the mean age of 44.57 ±3.44). The research process was conducted in a therapeutic-educational center of cancer. The sampling period was from November 2012 to late April 2013. The patients who had tumors were referred to departments. Also, patients expressed their conscious consents and their consents were confirmed by the hospital s ethical committee.

RESULTS

The data gathered from statistical samples are illustrated in the following table. Each patient s age & gender is demonstrated in the column next to them. The mean metabolites of each person were calculated with the precision of 1.1000 in Field Of View (FOV) and the Cho/Cr and Cho/NAA ratios were also calculated and presented in tables 1 and 2. Table 1 contains the patients mean metabolites and table 2 entails the metabolite density in control samples (the participants who had normal metabolite density). In table 3, the metabolite and disease intensity ratios were demonstrated in terms of Cho/Cr and Cho/NAA metabolite ratios.

T**able 1.** The findings related to the mean metabolite density in the statistical samples of the 30 patients.

NO	Age	Sex	Cho	Cr	NAA
1	11	M	1.08850	0.70500	0.63475
2	46	F	0.84977	0.52338	0.37483
3	59	F	0.47270	0.17330	0.24190
4	31	M	0.95314	0.34929	0.29700
5	70	M	1.54667	0.58777	0.28103
6	31	F	1.86200	1.05360	0.25695
7	29	F	0.37869	0.27131	0.34904
8	58	M	0.98725	0.30325	0.22875
9	17	M	0.47633	0.75767	0.39133
10	28	M	0.74175	0.40975	0.43850
11	30	F	1.34250	0.79750	0.22743
12	60	F	0.55600	0.41969	0.35888
13	49	F	0.64080	0.48127	0.47267
14	28	F	0.57942	0.17415	0.16693
15	47	F	0.26031	0.20492	0.24269
16	34	M	0.55380	0.35840	0.34870
17	32	F	0.97415	0.34546	0.15298
18	49	F	0.91045	0.38373	0.17825
19	58	F	0.14459	0.17691	0.19364
20	56	M	0.06610	0.03740	0.04445
21	81	M	0.70200	0.26400	0.27228
22	64	M	0.45891	0.36087	0.37725
23	49	M	0.35754	0.25392	0.19861
24	34	M	0.26065	0.24647	0.12879
25	25	M	0.19838	0.37029	0.29950
26	19	F	0.41146	0.46354	0.32002
27	49	F	0.32853	0,48611	0.37251
28	63	F	0.23490	0.14774	0.29449
29	30	F	0.38313	0.26125	0.22111
30	7	F	0.20791	0.33007	0.25310

Table 2. Findings related to the density of the metabolites and the metabolite ratio in control samples.

No	Age	Sex	Cho	Cr	NAA	Cho/NAA	Cho/Cr
31	49	M	0.043	0.060	0.108	0.72	0.40
32	43	F	0.077	0.088	0.087	0.88	0.89
33	38	M	0.010	0.010	0.024	1.00	0.42
34	40	F	0.041	0.036	0.052	1.14	0.79
35	45	M	0.025	0.040	0.057	0.63	0.44
36	51	F	0.080	0.095	0.155	0.84	0.52
37	46	f	0.034	0.050	0.064	0.68	0.53

Table 3. Findings related to Cho/Cr and Cho/NAA metabolite rations in the statistical samples of the patients having tumors.

NO	Cho\NAA	Cho\Cr	Grade
1	1.71	1.54	Low
2	2.27	1.62	Low
3	1.95	2.73	High
4	3.21	2.73	High
5	5.50	2.63	High
6	7.25	1.77	High
7	1.08	1.40	Low
8	4.32	3.26	High
9	1.22	0.63	Low
10	1.69	1.81	Low
11	5.90	1.68	High
12	1.55	1.32	Low
13	1.36	1.33	Low
14	3.47	3.33	High
15	1.07	1.27	Low
16	1.59	1.55	Low
17	6.37	2.82	High
18	5.11	2.37	High
19	0.75	0.82	Low
20	1.49	1.77	Low
21	2.58	2.66	High
22	1.22	1.27	Low
23	1.80	1.41	Low
24	2.02	1.06	Low
25	0.66	0.54	Low
26	1.29	0.89	Low
27	0.88	0.68	Low
28	0.80	1.59	Low
29	1.73	1.47	Low
30	0.82	0.63	Low

The question which arises during testing and comparing the means of a healthy & a patient statistical community (student test) was whether two calculated metabolic ratios indicate a significant difference between the means of a healthy and a patient statistical community or not?. In order to investigate this question, T-student means Test was used. In this test, the zero hypotheses are that there is no significant difference between the means of the patient and leathery community and the opposite hypothesis puts emphasis on the existence of significance. If sig ≤ 0.05 the test will be valid & the mean is in the range of the low and the high limit which means the means differ significantly. The comparison between the two communities was conducted low-grade healthy- patients brain lumps. The results of T-student Test for the two independent groups & comparing their means indicate that the mean differences with 0.95 of the community are in the range of the recognized low & high limits, therefore, the zero hypothesis is rejected and there is a significant difference between the two metabolic ration in high and low-grade patients and the healthy people. Figure one illustrates the color metabolic map and the HMRS spectrum of one of the patients.

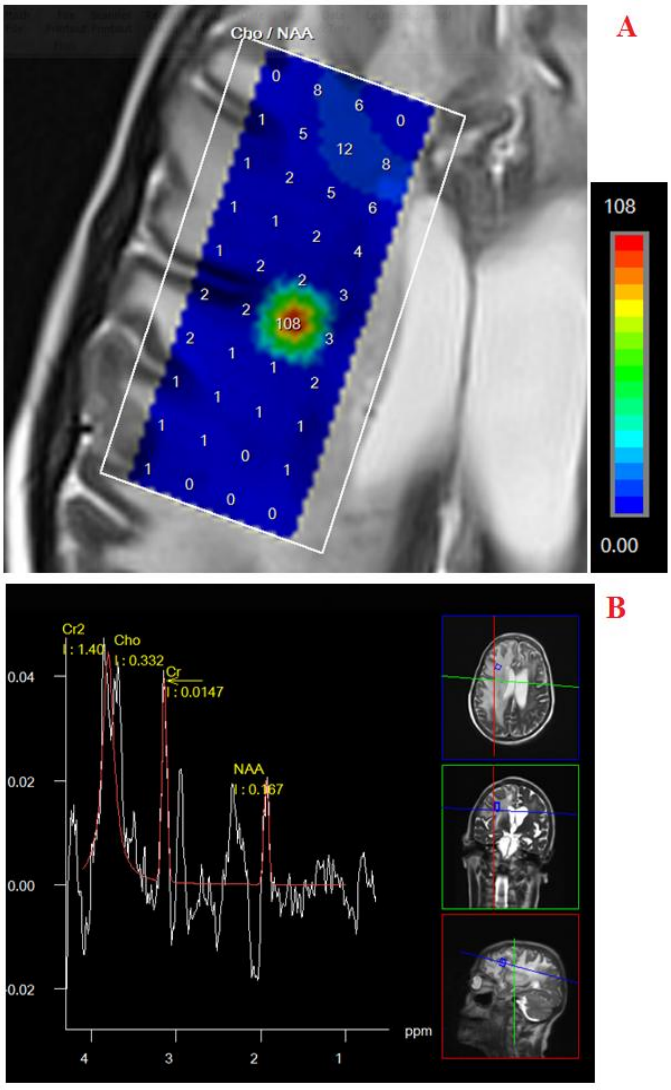

Figure1. A: A color metabolic d woman: the Cho/NAA in the highest ratio equals 108 which are illustrated in red and the lowest one equals zero. Which is shown in blue? The color metabolic mab in FOV equals zero and the Vexel is exactly placed on the toll. B: the metabolic density diagram which is resulted from a single-Vexel MRS. The relative reduction of density in NAA equals 0.167 & in Cho equals 0.323; the Cratine density equals 0.0147, FOV: 0.0, VOX: 1121.

DISCUSSION

The findings of this research show that NAA density has changed in the range of 0.044-0.635 with the mean of 0.13±0.2872. Cr densities has changed from 0.37 to 1.054 with the mean of 0.48± 0.38993 and the Cho density has changed from 0.066 to 1.862 with the mean of 0.184± 0.63094. The increase in Cho/NAA was observed to be from 0.660 to 7.0250 with the mean of 1.220± 2.4220 and that in Cho/Cr was observed to be from 0.540 to 3.330 with the mean of 0.627± 1.6860 in the brain lumps. Also, in comparing the independent healthy community with the patient one, sig ≤ 0.05 and there found to be a significant difference between these two communities the results of this study are in line with Alger JR and et al findings in their 1990 study in which the Hydrogen (protein) spectroscopy was successfully verified for distinguishing malignant brain tumors from the normal brain tissue both in adult an in children[12]. In their study in 2009; Tina Young Puissant and et al, found that Cho signal has a linear connection with the cellular density (opposite to what is observed with the obvious proliferative index). Instead of proliferative index, Cho peak is higher in the center of neoplastic mass and decreases environmentally. Cho signal is always low in the necrosis areas [13]. In evaluating the metabolite densities, in the center of the lumps and it is clearly observable in the color metabolite map of some patients (figure 1). These increases are linearly related to the cellular density. Since galliumare naturally proof read which destroy the neurons and decrease the NAA, therefore, cells tend to be tumoral. In 2005, Colen and et al found the decrease of NAA of the whole brain to be higher than the main tumor in the patients suffering from glioma tumors. This significant de chine of NAA of the whole brain probably indicates the extensive penetration of the tumor in the seemingly-healthy brain in MRI[14]. In an MRS quantitative study in 2006; Stadtbauer and et al found a correlation between the percentage of the tumor penetration of the MRS-guided biopsy samples and the changes of NAA, Cho and Cho/NAA ratios in the related Vexels[15]. In 2009, Soares DP, law found that the net density of NAA decreases with the tumor proliferative index, while the net Cho density and Cho/NAA ratio increases with that[16]. We had the same findings in our studies. In the cases where there was a high decrease of NAA in the patients' metabolite density, it was revealed that these patients have high-rate glioma.

The lumps with high-grade of malignancy (3-grade anaplastic gallium and Multiforme Glioblestoma or 4-grade ones) contain higher Cho and lower NAA compared to the low-grade lumps. Increased cho is associated with duplication and cellular density. In a systemic study conducted by Tlolling Worth et al; in 2006, it was reported that MRS is able to precisely distinguish high and low grade glioma. However, grading gliomas using MRS is really expanded diverse. This expanded diversity might be the result of different methods as well as different metabolites which could not make a difference among statistical overlapping in various grades of tumor, significant Cho/Cr and Cho/NAA and the high CBV in the grading of the glioma[17].

Using MRS, Smith JK and et al (2008) and,Lee and et al (2000) have found that the brain tumors show the increased levels of Cho and the decreased levels of NAA compared to those in the normal brain and reflect the rise in cellular tissue[18,19]. Furthermore Licavcanva andet al (2005) discovered that the increase in Cho is the indicator of the change & transformation of the cellular membrane the Cho has its peak correlation with mitotic activity and the malignancy grade of the tumor, so that the total Cho increase is along with the development of the tumor[20]. Our finding in the present study is in accordance with theirs. Another change in brain tumors is NAA decrease.

This metabolite is a nervous marker whose decrease demonstrates the destruction and displacement of the normal tissue. Lack of NAA in inter-axial tumor shows that either the source is somewhere outside the central nervous system (metastase) or that the tumor is so malignant that destroyed all the neurons in that area[21].

On the other hand, Cr signal is a little capricious in brain tumors and changes according to the type and grade of the tumor. H-MRS a typical spectrum for a brain tumor has a high-level Cho, low NAA and a little change in cr. Our finding in the present study also indicates the same changes.

Usually Cho increase is associated with an increase in the Cho/NAA or Cho/Cr ratios compared to its net density. Although estimating the net dandify of Cho, it is sensitive to several errors that are essential to hypothesize. Therefore, Cho/NAA and Cho/Cr ratios are more precise to study. Cho-increase is observed in all the neoplastic destruction. The Cho peak can help with the diagnosis, preventing its development as well as curing the tumor. This increase is due to its participation in the cellular membrane s turnover which reflects cellular multiplication.

Spaminato and et al (2007) discovered that it might be possible that low-grade Oligodendroglioma signify the increased Cho whichinfect, imitate the high grade tumor. These low grade tumors have high cellular density but lack multiplication and are telliahic and necrosis [22]. Reviewing the previous related articles demonstrate that those differences regarded in the spectroscopy technique, such using TE and choosing a method to specify the metabolite ratios are considered to show the differences in Cho levels between low and high grade gliomas and also the low gread gliomas indicate lower levels of Cho than the high grade ones[23]. This difference is ever understood using various methods of Cho quantitative assessment the tumor proton MRS clinical applications in grading gliomas are still under study at this stage, it is really important to understand the fact that MRS is sensitive to metabolic changes. Clinically, it is not unusual to find some low grade glioma with very high Cho:Cr &Cho:NAA ratios or the reverse high grade glioma with lower Cho:Cr &Cho:NAA ratios which is in the first place, due to the expanded necrosis. There are some overlaps among tumors with different grades. This also might be attributed to the various uses of metabolite ratios rather than an absolute quantitative assessment for comparing the grades of glioma.

CONCLUSION

In tumors, anytime the synthesis of the membrane and multiplication increase, Cho level increases too; while, NAA levels decline, because neurons & axons destroy and demolish. In addition, there is a decline in Cr levels in tumors because of the exhaustion and increase of lipids in necrosis. MRS metabolite ratios (Cho/Cr &Cho/NAA) can be applicable to grade and distinguish the tumors. 37 patients have been studied in terms of assessing their brain tumor metabolite. Among them, a comparison was conducted between 30 ones who showed NAA decrease and tumoral Cho increase and the other 7 who showed normal metabolite density.

They have also been studied in terms of tumor grading based on WHO grading system and Cho/NAA &Cho/Cr ratios were supposed to be the grading standards. 20 out of these 30 tumoral patients were low grade and the other 10 were high grade.

The results of this study demonstrated that high grade gliomas have higher mean Cho/NAA &Cho/Cr ratios than the low grade ones. Based on the reported results from patient'smagmatic resonance spectroscopy and comparing them to the control samples, it can be said that the magnetic resonance spectroscopy is efficient in assessing brain tumor metabolites and grading the tumors; therefore, it should be considered as a part of clinical diagnosis.

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