^{1}H MAGNETIC RESONANCE SPECTROSCOPY ASSESSMENT OF THE TEMOZOLOMIDE RESPONSE IN PEDIATRIC PILOCYTIC ASTROCYTOMAS

Arturo Alvarado[1,2], Agusto Pereira[3], María Cecilia Gómez[3], Rafael Cano[1]

ABSTRACT

Pilocytic Astrocytomas (PA) treatment and prognosis is variable depending on location. ^{1}H Magnetic Resonance Spectroscopy (MRS) is used to characterize tumor metabolism providing additional information to the Magnetic Resonance Imaging (MRI) evaluation assessing the therapy response. This study was designed to evaluate brain metabolic changes that result from Temozolomide (TMZ) administration on pediatric PA using MRS. Twenty children with PA were studied. We performed MRI and MRS pretreatment and after 12 months of therapy on a 3.0 Tesla scanner in order to monitor the chemotherapy response to 5-day treatment with oral TMZ (200 mg/mt² x day) given every 28 days for 12 cycles. Multivoxel Proton Spectroscopic Imaging was performed using a Point Resolved Spectroscopy sequence (PRESS). Analysis of Variance (ANOVA) was applied to the results in order to evaluate the possible statistical differences. Pairwise comparisons with Bonferroni correction test were assessed in order to verify the differences among ratio means. It was observed a significant decrease in Cho/Cr ratio ($p<0.05$) and a significant increase in NAA/Cr ratio ($p<0.05$) while TMZ therapy was taking place. These results are linked with tumor size reduction ($r = 0.95$, $p< 0.05$) detected by MRI. Results show MRS can detect early tumor reaction to therapy prior to MRI. Therefore, MRS could provide a useful tool to monitor the answer of pediatric PA to TMZ. The link between metabolic markers changes due to TMZ treatment assessed by MRS and the tumor volume reduction may also provide a fertile ground to develop a TMZ-based therapy for pediatric PA and to predict its efficacy to improve PA's response to treatment.

Key words: *^{1}H Magnetic Resonance Spectroscopy, Pilocytic Astrocytoma, Temozolomide.*

Address for correspondence: arturoalvaradopisani@gmail.com, arturo.alvarado@ucv.ve
Phone: 58-212-6050654 / 58-414-2236694

1 Arturo Alvarado. Doctor en Ciencias Fisiológicas, Mención Bioquímica (1990). Curso de Verano Postdoctoral, Universidad de Tübingen, Alemania (2002). Magíster Scientiarum en Farmacología (1984). Profesor Asociado. Universidad Central de Venezuela, Facultad de Medicina, Escuela "Luis Razetti, Cátedra de Farmacología y Toxicología, Instituto de Medicina Experimental. Coordinador Administrativo y de Actualización Tecnológica.

2 Investigador, CDD, Centro de Diagnóstico Docente "Las Mercedes".

3 Augusto Pereira, Médico Cirujano. Postgrado en Oncología. Postgrado en Pediatría. Jefe del Servicio de Oncología Pediátrica. Hospital de Niños "JM de Los Ríos". Caracas, Venezuela. María Cecilia Gómez. Médico Cirujano. Postgrado en Pediatría. Postgrado en Oncología Pediátrica. Hospital de Niños "JM de Los Ríos". Arturo Alvarado. Servicio de Pediatría Oncológica, Caracas, Venezuela.

2 Arturo Alvarado. Rafael Cano. Técnico Superior Universitario en Radiología e Imagenología. Centro de Diagnóstico Docente "Las Mercedes".

INTRODUCTION.

Pilocytic Astrocytomas (PA) represents 6 % of all brain tumors in humans while in childhood is approximately 15 % (1). They are the most common astrocytic tumors in children, accounting for 80-85 % of cerebellar astrocytomas and 60 % of optic gliomas (1-2). Juvenile PA usually arises in the cerebellum, brainstem, hypothalamic region, or optic pathways, but they may occur in any area where astrocytes are present, including the cerebral hemispheres and the spinal cord (3). These tumors are usually discrete, indolent lesions associated with cyst formation. The cysts may be unilocular or multilocular, with an associated tumor nodule. The most common presenting symptoms are associated with increased intracranial pressure resulting from mass effect or hydrocephalus. Symptoms may include headache, nausea, vomiting, irritability, ataxia, and visual complaints depending on the site of occurrence (3). The treatment and prognosis is variable depending on location (4). Temozolomide (TMZ) is an imidazole tetrazinone and an orally active alkylating agent (5-6). The drug is particularly useful in patients with brain tumors due to its excellent penetration into the Central Nervous System and almost 95-99 % bioavailability and linear pharmacokinetics after oral administration (7-13). ^{1}H Magnetic Resonance Spectroscopy (MRS) is used to characterize tumor metabolism providing additional information to the MRI evaluation to assess the therapy response (14-20). This study was designed to evaluate brain metabolic changes that result from TMZ administration on pediatric PA using MRS.

METHODS.

Twenty children with PA were studied after approvals of their parents and the Ethics Committee of the CDD were obtained. We performed MRI and MRS pretreatment and after 12 months of therapy on a 3.0 Tesla scanner (Signa Excite®, GE Medical Systems) in order to monitor the chemotherapy response to 5-day treatment with oral TMZ (200 mg/mt² x day) given every 28 days for 12 cycles (5-13). 2D-Multivoxel Proton Spectroscopic Imaging was performed using a Point Resolved Spectroscopy sequence (PRESS) with fixed parameters at TR = 1500 ms, TE = 35 ms, FOV = 20 cm, phase 16, section thickness 10 mm and acquisition time 9' 41" (14-15). The spectroscopic data set was spatially zerofilled to 32 x 32 images providing a final voxel resolution of 0.75 cm x 0.75 cm x 1.00 cm = 0.56 cm³. Metabolic signals of Lipids (Lip), Lactate (Lac), N-Acetylaspartate (NAA), Creatine (Cr), Choline (Cho) and myo-Inositol (mI) were detected and NAA/Cho, NAA/Cr, Cho/Cr and mI/Cr ratios were calculated. Analysis of Variance (ANOVA) was applied to the results. Pairwise comparisons with Bonferroni correction test were assessed in order to verify the differences among ratio means.

RESULTS.

The Table 1 display the data obtained from the selected voxel in each patient enrolled in the study. The values represent the ratios referred to Cr (NAA/Cr, Cho/Cr and mI/Cr).

TABLE 1. Individual data sets for metabolic ratios.

ID	NAA/Cr pre	NAA/Cr post	Cho/Cr pre	Cho/Cr post	mI/Cr pre	mI/Cr post
1	0,89	1,09	1,41	1,09	1,16	1,12
2	0,95	1,14	1,71	1,28	1,42	1,36
3	0,76	0,97	1,92	1,45	1,53	1,46
4	0,84	1,12	1,48	0,85	0,98	0,90
5	0,70	0,97	1,56	0,97	0,80	0,74
6	1,11	0,99	1,00	1,18	1,22	1,16
7	1,35	1,47	1,03	1,25	1,21	1,15
8	0,86	1,13	1,77	1,20	0,84	0,79
9	0,83	1,12	1,93	1,28	1,18	1,10
10	1,19	1,62	1,54	0,67	0,63	0,55
11	1,16	1,35	1,63	1,29	1,58	1,53
12	1,52	1,85	1,83	1,45	1,82	1,76
13	1,16	1,45	1,60	1,17	1,73	1,68
14	1,06	1,32	1,47	1,09	1,58	1,54
15	0,86	1,12	1,54	1,26	2,01	1,96
16	1,04	1,28	1,44	1,15	1,32	1,27
17	1,57	1,81	1,59	1,15	1,70	1,63
18	0,96	1,19	1,13	0,92	0,99	0,96
19	0,90	1,22	1,35	1,10	1,37	1,33
20	1,66	1,89	1,52	0,95	1,79	1,70
μ ± SD	1.07 ± 0.27	1.31 ± 0.29	1.52 ± 0.26	1.14 ± 0.19	1.34 ± 0.37	1.28 ± 0.38

NAA = N-Acetylaspartate, Cr = Creatine, Cho = Choline, mI = myo-Inositol, ID = patient identification number, pre = previous to receive treatment with TMZ, post = after 12 months of TMZ therapy, μ = mean, SD=Standard Deviation

Figure 1 illustrates the metabolic signals intensities changes as a consequence of chemotherapy administration. We clearly note the reduction in the Cho/Cr ratio and the NAA/Cr ratio increase observed between the two subsequent MRS. The MRS profile obtained previous to treatment start (A) demonstrate a mI high intensity signal which has been described previously in Astrocytomas.

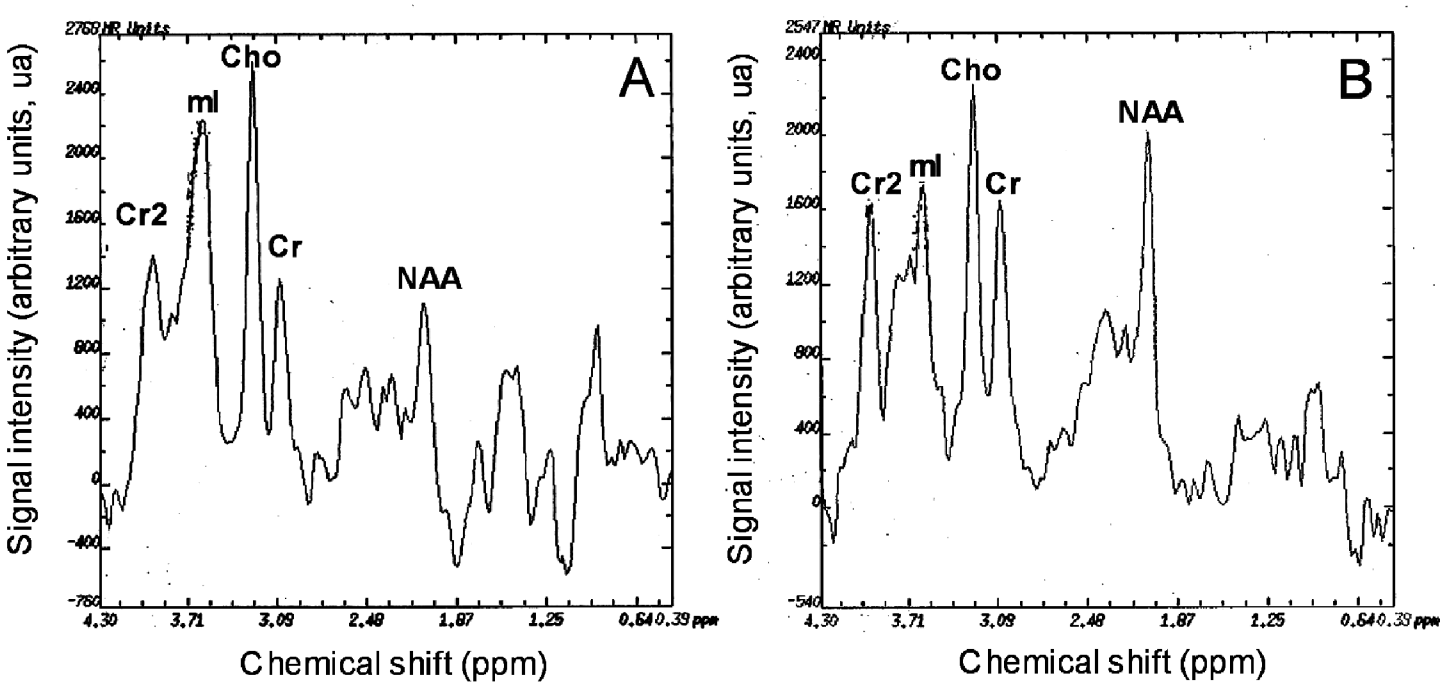

Figure 1. Comparison between the MRS profiles obtained previous of the TMZ treatment start (A) and 12 months after chemotherapy administration (B).

Figure 2 is a composed image that shows the contrast media enhancement of a PA at the sagittal, coronal and axial views of the MRI study in combination with Multivoxel MRS Chemical Shift Imaging performed in patient number 6 obtained from Cho/Cr ratio at axial projection. This child shows a progression of the disease probably caused by unresponsive to TMZ treatment. Note in Table 1 that NAA/Cr ratio decrease and Cho/Cr ratio rise in this subject between the two observations

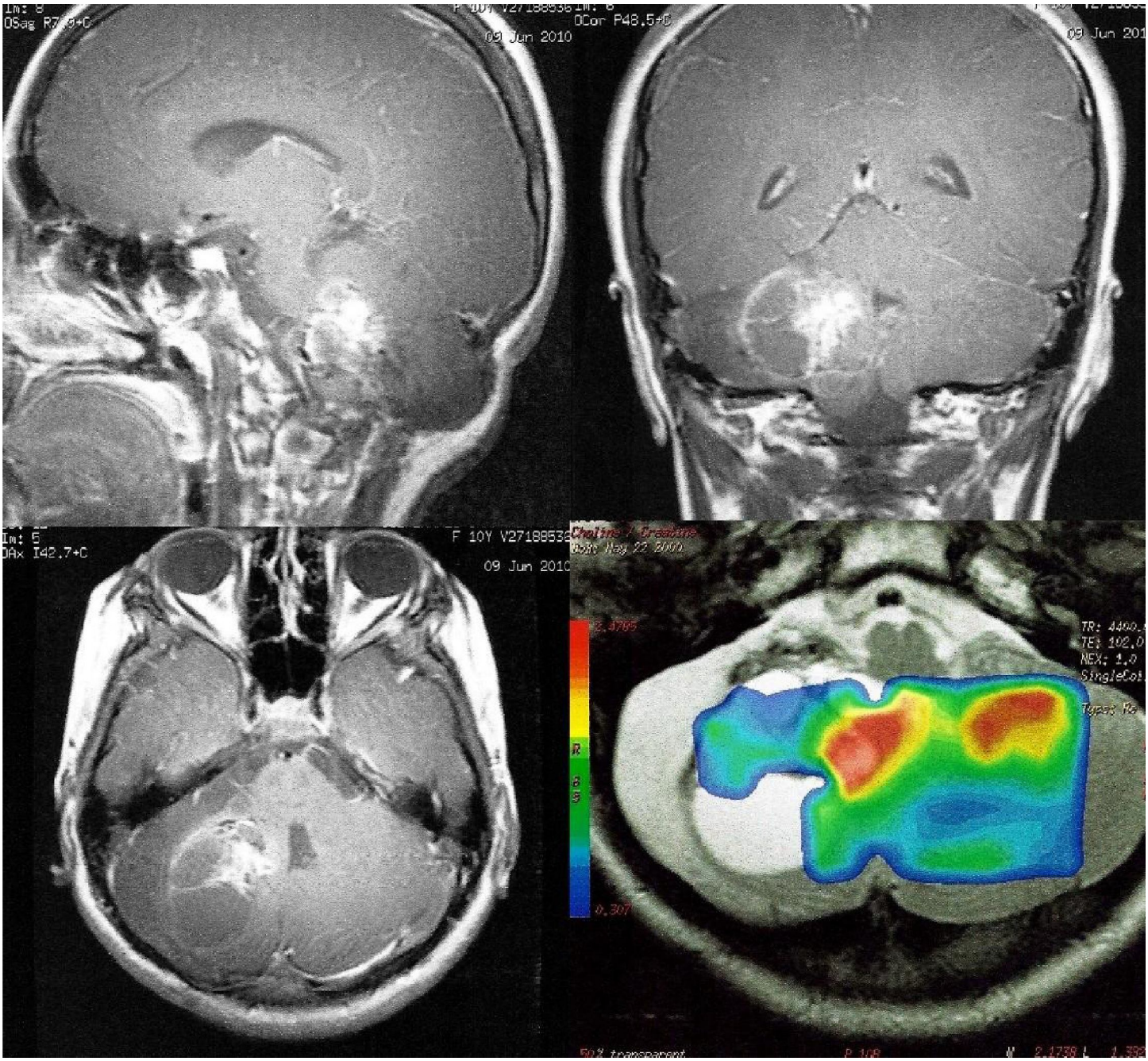

Figure 2. Composed image (sagittal, coronal and axial MRI planes) grouping with CSI perform in patient number 6. The red over yellow color demonstrate the highest Cho/Cr ratio regions. Note that the red color are present at the right side of cerebellum which is the original location of the lesion and at the left side suggesting a discrete tumor activity which is not evident at the MRI study. The contrast enhancement is observed at the right side but not at the left.

DISCUSSION.

This study was capable to demonstrate the added value that MRS represents when it is performed simultaneously with MRI based on biochemical changes inspection. The individual values and averages plus standard deviation of metabolic ratios are show in Table 1. It can be detect a significant increase in NAA/Cr ratio, 1.07 ± 0.27 pre-TMZ in comparison with 1.31 ± 0.29 post-TMZ ($p<0.05$) and a significant decrease in Cho/Cr ratio, 1.52 ± 0.26 pre-TMZ matched with 1.14 ± 0.19 post-TMZ ($p<0.01$). The decline of Cho/Cr ratio could be a sign of tumor response whereas the signal reduction of choline-containing compounds may reflect a diminish cell membrane disruption and fewer alterations of the phospholipids metabolism which generally take place when cell growth inhibition is induced by antineoplastic drugs (21). The NAA/Cr increase may be a sign of a tumor environment improvement noticeable by reduction in solid tumor size and edema (22-27). The mI/Cr proportion does not statistical differ between the two observations.

The Figure 1 illustrates the MRS spectral pattern obtained in child number 10 classified as responder patient. In the image it can be distinguished a reduction in intensity signal of mI and Cho together with a NAA intensity signal augmentation as a result of the TMZ therapy between the two observations.

Patient number 6 do not react satisfactory to therapy as was catalogued as non-responder. In this case as can be observe in Table 1, NAA/Cr ratio decrease while Cho/Cr ratio increase 12 months after TMZ administration. This metabolic information demonstrate connection with the MRI results as is illustrated in Figure 2, in which can be confirmed the correspondence between the patterns of contrast media enrichment with the metabolic image obtaining from CSI (Cho/Cr) localized at the posterior cranial fossa. The image illustrated the PA residual activity at the right lobe of cerebellum and an extension of the lesion to the left lobe that can not be confirmed by MRI inspection alone without the MRS examination. Thus, the study was capable to demonstrate the added value that MRS represents when it is performed simultaneously with MRI. Although Computed Tomography and MRI are the primary problem-solving imaging methods for initial diagnosis of brain tumors before surgery, MRS may further increase the diagnostic accuracy, confidence and also may provide prognostic information (28-29). Tumor histology continues to be the most important prognostic factor to establish the type of treatment after surgery but biochemical parameters may have an essential role in determining the growth rate of tumors independently of histological grade (30). MRS may also contribute to establish a prognosis in early stages of the disease opening a space to improve the therapeutic response to TMZ making it more effectively with fewer side effects (1-6). Multivoxel MRS allows the compilation of spectral data from multiple regions, including the tumor itself and its surroundings given information about the possible cancerous activity present at solid component, edema, penumbra regions and cystic portion of the PA allowing an accurately differentiation between tumor tissue from radiation necrosis, normal tissue, or other structural abnormalities that can not be determined with MRI examination alone (31-33). We suggest that MRS can detect early tumor reaction to therapy prior to MRI. Therefore, MRS may provide a useful tool for monitoring the answer of pediatric PA to TMZ. The link between metabolic markers changes due to TMZ treatment assessed by MRS and the tumor volume reduction may also provide a fertile ground to develop a TMZ-based therapy for pediatric PA and to predict its efficacy to improve PA's response to treatment.

REFERENCES.

1. Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 2005; 64:479-489.

2 Kaderali Z, Lamberti-Pasculli M, Rutka J. The changing epidemiology of paediatric brain tumours: a review from the Hospital for Sick Children. Childs Nerv Syst 2009; 25:787-793.

3. Goetz C, Peraud A, Kreth F, Tonn J. Pilocytic astrocytoma in childhood. MMW Fortschr Med 2004; 146:24-25.

4. Qaddoumi I, Sultan I, Broniscer A. Pediatric low-grade gliomas and the need for new options for therapy: Why and how? Cancer Biol Ther 2009; 8:4-10.

5. Chiang K, Chang K, Lee Y, Huang P, Hsu T, Chen Y, Chang F, Wong T. Role of temozolomide in the treatment of newly diagnosed diffuse brainstem glioma in children: experience at a single institution. Childs Nerv Syst 2010; 26:1035-1041.

6 Wang C, Hsu T, Wong T, Chang K. Efficacy of temozolomide for recurrent embryonal brain tumors in children. Childs Nerv Syst 2009; 25:535-541.

7. Khaw S, Coleman L, Downie P, Heath J, Ashley D. Temozolomide in pediatric low-grade glioma. Pediatr Blood Cancer 2007; 49:808-811.

8. Gururangan S, Fisher M, Allen J, Herndon J, Quinn J, Reardon D, Vredenburgh J, Desjardins A, Phillips P, Watral M, Krauser J, Friedman A, Friedman H. Temozolomide in children with progressive low-grade glioma. Neuro Oncol 2007; 9:161-168.

9. Baruchel S, Diezi M, Hargrave D, Stempak D, Gammon J, Moghra bi A, Coppes M, Fernandez C, Bouffet E. Safety and pharmacokinetics of temozolomide using a dose-escalation, metronomic schedule in recurrent paediatric brain tumours. Eur J Cancer 2006; 42:2335-2342.

10. De Sio L, Milano G, Castellano A, Jenkner A, Fidani P, Dominici C, Donfrancesco A. Temozolomide in resistant or relapsed pediatric solid tumors. Pediatr Blood Cancer 2006; 47:30-36.

11. Aryan H, Meltzer H, Lu D, Ozgur B, Levy M, Bruce D. Management of pilocytic astrocytoma with diffuse leptomeningeal spread: two cases and review of the literature. Childs Nerv Syst 2005; 21:477-481.

12. Kuo D, Weiner H, Wisoff J, Miller D, Knopp E, Finlay J. Temozolomide is active in childhood, progressive, unresectable, low-grade gliomas. J Pediatr Hematol Oncol 2003; 25:372-378.

13. Zheludkova O, Tarasova I, Gorbatykh S, Belogurova M, Kumirova E, Borodina I, Prityko A, Melikian A, Shcherbenko O. Treatment of anaplastic astrocytomas and glioblastomas in children by the use of temozolomide (TMZ). Vopr Onkol. 2002; 48:356-360.

14. Wang Z, Sutton L, Cnaan A, Haselgrove J, Rorke L, Zhao H, Bilaniuk L, Zimmerman R. Proton MR spectroscopy of pediatric cerebellar tumors. Am J Neuroradiol 1995; 16: 1821-1833.

15. Hwang J, Egnaczyk G, Ballard E, Dunn R, Holland S, Ball W Jr. Proton MR spectroscopic characteristics of pediatric pilocytic astrocytomas. Am J Neuroradiol 1998; 19: 535-540.

16. Panigrahy A, Krieger M, Gonzalez-Gomez I, Liu X, McComb J, Finlay J, Nelson M Jr., Gilles F, Blüml S. Quantitative Short Echo Time 1H-MR Spectroscopy of untreated pediatric brain tumors: preoperative diagnosis and characterization. Am J Neuroradiol 2006; 27: 560-572.

17. Harris L, Davies N, MacPherson L, Foster K, Lateef S, Natarajan K, Sgouros S, Brundler M, Arvanitis T, Grundy R, Peet A. The use of short-echo-time 1H MRS for childhood cerebellar tumours prior to histopathological diagnosis. Pediatr Radiol 2007; 37:1101-1109.

18. Panigrahy A, Nelson M, Finlay J, Sposto R, Krieger M, Gilles F, Blüml S. Metabolism of diffuse intrinsic brainstem gliomas in children. Neuro- Oncol 2008; 10: 32-44.

19. Harris L, Davies N, MacPherson L, Lateef S, Natarajan K, Brundler M, Sgouros S, English M, Arvanitis T, Grundy R, Peet A. Magnetic resonance spectroscopy in the assessment of pilocytic astrocytomas. Eur J Cancer 2008; 44: 2640-2647.

20. Curless R, Bowen B, Pattany P, Gonik R, Kramer D. Magnetic resonance spectroscopy in childhood brainstem tumors. Pediatr Neurol 2002; 26: 374-378.

21. Tzika A, Cheng L, Goumnerova L, Madsen J, Zurakowski D, Astrakas L, Zarifi M, Scott R, Anthony D, Gonzalez R, Black P. Biochemical characterization of pediatric brain tumors by using in vivo and ex vivo magnetic resonance spectroscopy. J Neurosurg. 2002; 96: 1023-1031.

22. Porto L, Kieslich M, Franz K, Lehrbecher T, Vlaho S, Pilatus U, Hattingen E. Spectroscopy of untreated pilocytic astrocytomas: do children and adults share some metabolic features in addition to their morphologic similarities? Childs Nerv Syst 2010; 26:801-806.

23. Weybright P, Maly P, Gomez-Hassan D, Blaesing C, Sundgren P. MR spectroscopy in the evaluation of recurrent contrast-ehancing lesions in the posterior fossa after tumor treatment. Neuroradiology 2004; 46: 541-549.

24. Lazareff J, Olmstead C, Bockhorst K, Alger J. Proton magnetic resonance spectroscopic imaging of pediatric low-grade astrocytomas. Childs Nerv Syst 1996; 12: 130-135.

25. Schneider J, Confort-Gouny S, Viola A, Le Fur Y, Viout P, Bennathan M, Chapon F, Figarella-Branger D, Cozzone P, Girard N. Multiparametric differentiation of posterior fossa tumors in children using diffusion-weighted imaging and short echo-time 1H-MR spectroscopy. J Magn Reson Imaging 2007; 26: 1390-1398.

26. Schneider J, Viola A, Confort-Gouny S, Ayunts K, Le Fur Y, Viout P, Bennathan M, Chapon F, Figarella-Branger D, Cozzone P, Girard N. Infratentorial pediatric brain tumors: the value of new imaging modalities. J Neuroradiol 2007; 34: 49-58.

27. Arle J, Morriss C, Wang Z, Zimmerman R, Phillips P, Sutton L. Prediction of posterior fossa tumor type in children by means of magnetic resonance image properties, spectroscopy, and neural networks. J Neurosurg. 1997; 86: 755-761.

28. Hourani R, Horská A, Albayram S, Brant L, Melhem E, Cohen K, Burger P, Weingart J, Carson B, Wharam M, Barker P. Proton magnetic resonance spectroscopic imaging to differentiate between nonneoplastic lesions and brain tumors in children. J Magn Reson Imaging 2006; 23: 99-107

29. Zakrzewski K, Kreisel J, Polis L, Nowosławska E, Liberski P, Biegański T. Clinical application of proton magnetic resonance spectroscopy for differential diagnosis of pediatric posterior fossa tumors. Neurol Neurochir Pol 2001; 35 Suppl 5: 19-25.

30. Zakrzewski K, Kubicki M, Polis L, Nowosławska E, Liberski P. Proton magnetic resonance spectroscopy of primary pediatric brain tumors: neuropathological correlation. Folia Neuropathol 1999; 37: 148-151.

31. Lazareff J, Gupta R, Alger J. Variation of post-treatment 1H-MRSI choline intensity in pediatric gliomas. J Neurooncol. 1999; 41: 291-298.

32. Cecil K, Jones B. Magnetic resonance spectroscopy of the pediatric brain. Tops Magn Reson Imag 2001; 12: 435-452.

33. Warren K, Frank J, Black J, Hill R, Duyn J, Aikin A, Lewis B, Adamson P, Balis F. Proton magnetic resonance spectroscopic imaging in children with recurrent primary brain tumors. J Clin Oncol 2000; 18:1020-1026.