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Original Article

Correlation of Pre-Operative Myo-Inositol/Creatine Ratio with Histopathological Grade of Glioma

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Abstract

Background: *Introduction: Gliomas are the most frequent primary intracerebral tumors in adults & constitute 35–50% of all intracranial neoplasms. In determining a treatment plan, tumor grade is a key consideration for minimizing the risk of unnecessary morbidity and mortality.*

Objective: *To evaluate Correlation of pre-operative Myo-inositol/ Creatine ratio with histopathological grade of glioma.*

Materials and method: *This was a cross sectional experimental study. Total 33 patients consistent with selection criteria were enrolled in this study. Myo-inositol/ Creatine ratio was obtained from MRS. Histopathological grade of glioma was obtained from biopsy (hematoxylin and eosin stain or HE stain) report. The obtained results were then grouped categorically.*

Result: *Out of 33 patients, 22 were male and 11 were female. According to histopathology, 14 gliomas were confirmed as high grade and 19 gliomas were confirmed as low grade. Spearman Rank Correlation Test showed a moderate negative association between Myo-inositol/ Creatine ratio with glioma grading with coefficient value of r = -0.611 with a statistically significant p-value (p<0.001). We were able to differentiate between low grade (II) and high grade (III + IV) gliomas using the Myo-inositol/ Creatine ratio. The levels of MI/ Cr were higher (e"0.48) in patients with low-grade glioma, and lower (<0.48) in patients high grade glioma*

Conclusion: *This study has shown a significant correlation between pre-operative Myo-inositol/ Creatine ratio with histopathological grade of glioma. It may help neurosurgeons in taking clinical decisions about patient management and counselling.*

Keywords: *Glioma, MRI, MRS, grading, Myo-inosito, Creatine.*

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Introduction:

The term 'glioma' was coined by Virchow in 1863. These are primary brain tumors arising from glial cells. There are more than 100,000 cases of central nervous system (CNS) cancer diagnosed each year worldwide (Parkin 2000), and gliomas represent 40% of all brain tumors (Liang et al. 2020).

According to their aggressiveness, the World Health Organization (WHO) classifies gliomas into Grades 1 and 2 or low grade gliomas (LGG) and Grades 3 and 4 or high grade gliomas (HGG) (Ganau et al. 2015). Grade I applies to lesions with low proliferative potential and the possibility of cure following surgical resection alone. Neoplasms designated grade II are generally infiltrative in nature and, despite low-level proliferative activity, often recur. Those showing anaplasia and mitotic activity as grade III and tumors additionally showing microvascular proliferation and/or necrosis

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as WHO grade IV (WHO 2007). Grade is one component of a combination of criteria used to predict a response to therapy and outcome.

The current ‘‘gold standard’’ for the determination of glioma grade is by surgical biopsy/resection and histopathological assessment. However, biopsy approach may suffer from several sources of errors (Jackson et al. 2001), the most significant of which is limited number of samples thus creating potential errors in determining glioma grade. GBMs are known for having an extensively heterogeneous histopathology (Louis et al. 2016) which increases the risk of retrieving non-representative tumor samples for histological assessments. A substantial proportion of the assessed histological features are at risk of being underrepresented when tissue material is limited, including most of the grading features (Mikkelsen et al. 2020). As a result, a high-grade tumor may be diagnosed as low grade because the samples were taken at a less malignant region.

Alternatively, noninvasive or minimally invasive imaging technologies have been used to evaluate the malignancy of brain tumors (Lu et al. 2008). Contrast-enhanced magnetic resonance imaging (MRI), now a standard procedure for suspected brain tumor cases, can cover a large field of view with the advantages of high spatial resolution and relatively low invasiveness. However, this approach is relatively qualitative and does not provide a quantitative index for a direct measure of tumor grade.

In vivo magnetic resonance spectroscopy (MRS) is one of the MR methods that start to play an important role in determining most brain tumor types and grades. The principle of MRS is based on metabolite detection by measurement of the spectra of specific isotopes, e.g., 1H, 13C or 31P. Metabolites that can be identified on a standard brain proton MRS include N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), gamma-aminobutyric acid (GABA), aspartate (Asp), glycine (Gly), choline (Cho), creatine (Cr), phosphocreatine (PCr), glutamate (Glu), glutamine (Gln), myo-inositol (mIns), taurine (Tau), lactate (Lac), glucose (Glc), alanine (Ala), phenylalanine (Phe), histidine (His), lipids (Lip), and acetate. Concentrations of the metabolites are relatively steady for each specific “healthy” tissue but they may shift due to disturbances in metabolism during pathological states. Because of its noninvasive and safe nature, MRS has a great advantage in patients with brain gliomas, particularly if there are some contraindications for surgical procedure or significant risks due to location of tumor in eloquent areas or comorbidities of patients (Bulik et al. 2013).

Previous studies have shown the potential of MRS to differentiate low grade from high grade gliomas (Law et al. 2003). They used Cho/Cr, Cho/NAA ratios in the determination of the glioma grade. They had observed higher Cho/Cr and Cho/NAA in high grade compared to low grade tumors, though threshold values of metabolite ratios for grading of gliomas were not well established. However, high levels for Cho with high Cho/Cr and high Cho/NAA ratios had been observed in some low grade gliomas. This is in concordance with the previous report by law et al. (2003) who reported high Cho level in low grade glioma. On the other hand Hall et al. (2001) reported low Cho level ratios in some GBM. This may be due to extensive necrosis, which increases the false-positive rates and false-negative rates in predicting low and high grade gliomas, respectively (Soares and Law 2009).

Myo-inositol (mIns) is a simple sugar that can be described as marker of astrocytes or glial cell marker in the adult brain because it is synthesized by astrocytes and participates in their osmoregulatory system (Brand et al. 1993). It is likely that it also contributes to the maintenance of brain volume (Isaacks et al. 1994). With the help of MRS, mIns can be detected as a multiplet of peaks with main components located at 3.5 ppm of the spectrum. The elevation in mIns detected in the MR spectra can be found in cerebral diseases associated with marked astrogliosis (Hattingen et al. 2008).

Creatine (Cr) is called an energy metabolism marker that is synthesized from amino acids primarily in the kidneys and liver and transported to the peripheral tissues/organs by blood (Wyss and Kaddurah 2000). It is visualized in the MR spectra mainly as the high peak located at 3.0 ppm (Urenjak et al. 1993). Cr is a relatively constant element of cellular energetic metabolism of the brain and it is frequently used as a reference metabolite for in vivo MRS, e.g., for calculating metabolite ratios such as Cho/Cr, NAA/Cr, mIns/Cr or Lip/Cr (Verma et al. 2016).

The aim of this study was to see if there is any Correlation of pre-operative Myo-inositol/Creatine ratio with histopathological grade of glioma. This additional information provided by Magnetic Resonance spectroscopy (MRS) can be of high interest for taking

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clinical decisions about patient management and counselling.

Materials and Methods:

It was a cross sectional experimental study which was carried out in the Department of Neurosurgery, Bangabandhu Sheikh Mujib Medical University, Dhaka. It was conducted from September 2019 to March 2021 after the approval of the scientific and ethical committee.

The study population included all patients with glioma diagnosed by MRI with preoperative MRS with Myo-inositol/Creatine ratio & confirmed by histopathology (HE stain), admitted into Department of Neurosurgery, Bangabandhu Sheikh Mujib Medical University, Dhaka Medical College and Hospital and National Institute of Neuroscience and Hospital.

Purposive sampling technique was used. Patients, who fulfilled the selection criteria were selected for this study.Patient diagnosed as a case of gliomas on MRI who had preoperative MRS and Gliomas confirmed by histopathology (HE stain) were included in this study. On the other hand, patients with alzheimer disease and hepatic encephalopathy, histopathology report not consistent with glioma was excluded from our study.

Our demographic variables were age and sex, imaging variables were myo-inositol, creatine, myo-inositol/ creatine ratio and histopathological variables were low (Grade I and II) and high (Grade III and IV) grade glioma.

Results:

Table-I

Frequency Distribution of the Study Subjects according to Age (n=33)

Age (years)	Frequency (n)	Percentage (%)
≤20	4	12.1
21-30	9	27.3
31-40	9	27.3
41-50	6	18.2
51-60	5	15.2
Mean ±SD	35.27 ± 12.51 Years	

The age distribution of 33 patients are divided into five groups and is shown in the Table I. The age range of patients was 8 to 59 years. The Mean ± SD were 35.27 ± 12.51 years. Most of the patients (54.6%) were among 21-40 years of age.

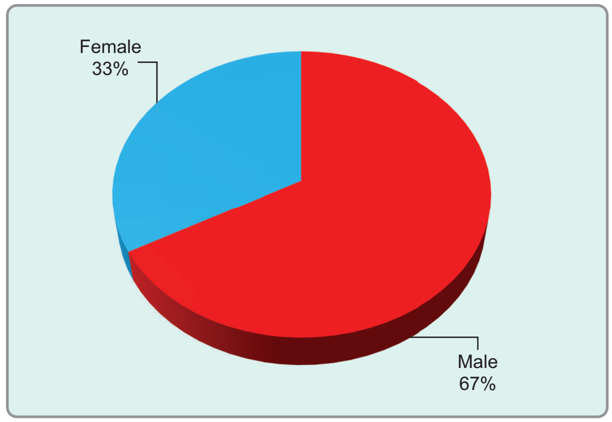

Figure 1: *Pie chart showing distribution of the study subjects according to gender.*

The gender distribution shows (Figure 7) out of 33 patients 22 patients (66.7%) were male and 11 patients (33.3%) were female. The male female ratio was 2:1.

Table-II

Grading of glioma (N=33)

	Frequency (n)	Percentage (%)
Histopathological grading of glioma		
Low Grade	19	57.5
High Grade	14	42.5

This table (Table II) shows most of the gliomas were low grade (57.5%)

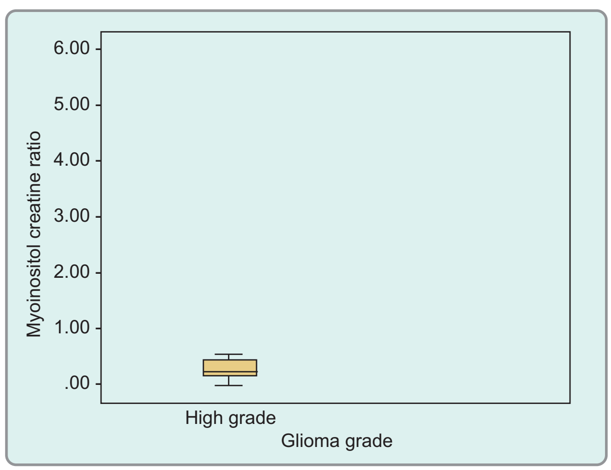

Figure 2: *Box plot of Myo-inositol/Creatine ratio in High grade of glioma (histopathological)*

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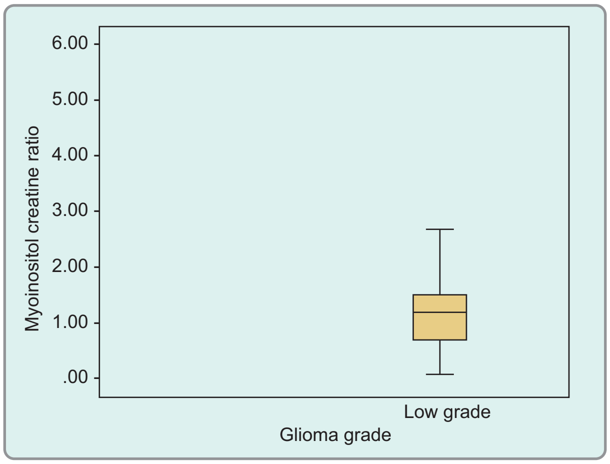

Figure 3: *Box plot of Myo-inositol/Creatine ratio in Low grade of glioma (histopathological)*

Boxplot chart is showing Myo-inositol creatine ratio is higher in low grade glioma when compared to high grade glioma

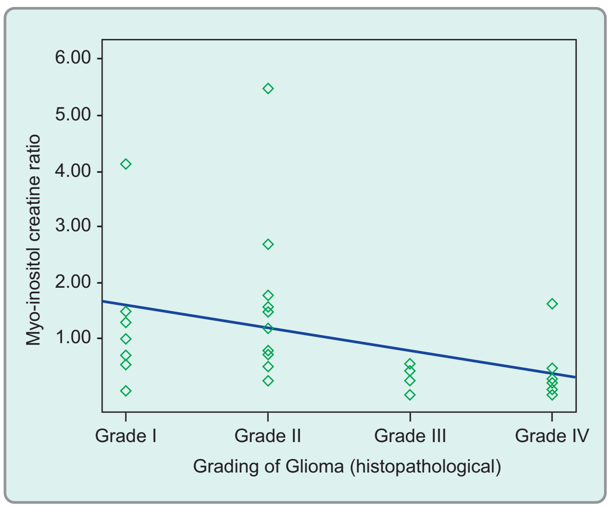

Figure 4: *Scattered diagram showing correlation of Myo-inositol creatine ratio with glioma grading (histopathological)*

Spearman Rank Correlation Coefficient Test was done for correlation of Myo-inositol creatine ratio with glioma grading (Figure 10). The test showed a moderate negative correlation. With correlation coefficient value of r = -0.611with a significant p-value of p = <0.001

Discussion:

Gliomas account for approximately 77% of primary malignant brain tumors and thus, they are most frequently diagnosed primary brain tumors (Schwartzbaum et al. 2006). Grade of glial tumor helps to determine the choice of therapy and predict prognosis (Bulik et al. 2013).

The current ''gold standard'' for the determination of glioma grade is by surgical biopsy/resection and histopathologic assessment. However, biopsy approach may suffer from several sources of errors (Jackson et al. 2001, Daumas-Duport et al. 1988), the most significant of which is limited number of samples thus creating potential errors in determining glioma grade. Also, every surgical procedure has a significant risk that can be expressed as mortality and morbidity (Bulik et al. 2013). A stereotactic brain biopsy is a careful minimally invasive procedure with low mortality (less that 1%), its overall morbidity about 3.5% is still significant (Hall 1999).

Non-invasive grading of gliomas is still considered a challenge (Chawla et al. 2007). Due to great advances in advanced magnetic resonance (MR) imaging methods, efficacy of this non-invasive diagnostic tool is increasing (Bulik et al. 2013). However, diagnosis of gliomas and their grading by conventional MR imaging is sometimes dubious due to sensitivity of glioma grading ranging from 55% to 83% (Law et al. 2003). Changes in the metabolism of tumor cells related to malignant transformation are reflected in changes of particular metabolite concentration in the tumor tissue (Horska and Barker 2010). Because glial tumors have some specific metabolite characteristics which further differ according to grade, there is a growing interest in MR spectroscopy that could further increase the sensitivity of routinely used diagnostic imaging (Bulik et al. 2013).

The clinical utility of proton MRS in glioma grading is still being investigated (Metwally et al. 2013). The results of glioma grading by using MRS vary widely which, may be attributed to different methods and metabolites overlapping between different tumor grades (Bertholdo et al. 2013).

Previous studies have shown the potential of MRS to differentiate low grade from high grade gliomas (Law et al. 2003). They used Cho/ Cr, Cho/NAA ratios in the determination of the glioma grade. They had observed higher Cho/Cr and Cho/NAA in high grade compared to low grade tumors, though threshold values of metabolite ratios for grading of gliomas are not well established. However high levels for Cho with high Cho/Cr and high Cho/NAA ratios had been observed in some low grade gliomas. This was in concordance with the previous report by law et al. (2003) who reported high Cho level in low grade glioma. On the other hand Hall et al. (2001) reported low Cho level ratios in some GBM. This may be due to extensive necrosis, which increases the false-positive rates and false-negative rates in predicting low and high grade gliomas, respectively (Soares and Law 2009).

In tthis study, we found significant association between pre-operative Myo-inositol/Creatine ratio with histopathological grade of glioma.

The mean age was 35.27 ± 12.51 years ranging from 8 – 59 years. Out of 33 patients, 22 were male and 11 were female with male to female ratio 2:1 which was consistent with previous studies but our ratio was slightly higher probably due to lower number of cases (Metwally et al. 2013).

We measured Myo-inositol/Creatine ratio from 1H-MRS. Low and high grade of glioma was confirmed from histopathology (Winn 2011). Spearman's Rank Correlation Coefficient Test showed negative association between Myo-inositol/Creatine ratio and grade of glioma with coefficient value of r = -0.611 at p-value ($p<0.001$) which is consistent with previous studies (Castillo et al. 2000, Metwally et al. 2013).

We were able to differentiate between low grade (II) and high grade (III + IV) gliomas using the Myo-inositol/ Creatine ratio. The levels of MI/Cr were higher (e"0.48) in patients with low-grade glioma, and lower (<0.48) in patients high grade glioma. This was an agreement with a previous study which reported that MI at short TE provided some separation between low grade astrocytoma and anaplastic astrocytoma (Majos et al. 2004).

However, our results disagreed with the previous reports by Kousi et al. (2012) who used 3T 1H-MRS in grading cerebral gliomas at short and long TE in 71 patients with untreated glioma. MI was observed to be increased for both glioma grades, the MI/Cr ratio was 0.85 ± 0.24 and 0.90 ± 0.35 for low and high grade gliomas, respectively and hence that ratio did not significantly differentiate the two tumor groups.

Kim et al. (2006) also used 3T MR-spectroscopy for the grading of glioma in 35 patients. They found that MI/Cr ratio increased with grade of the tumor with the MI/Cr ratio being 0.86 ± 0.19, 1.23 ± 0.37, 1.15 ± 0.52 for grade II, grade III, and grade IV tumors, respectively.

In this study the accuracy of Myo-inositol/Creatine ratio for predicting the glioma grade was 87.9%. Two cases were diagnosed as high grade glioma based on histopathology with high (e"0.48) Myo-inositol/ Creatine ratio. Another two low grade glioma had low Myo-inositol/Creatine ratio (<0.48). This may be explained by that the biopsy was not necessarily taken within the area of the lesion with greatest cellularity, so it may underestimate the tumor grade (Metwally et al. 2013, Smith et al. 2003) or the voxel site that was not at the same location of the biopsy site.

The limitations of this study includes short period of time with a small sample size. Only 33 cases were selected for statistical analysis which may bias the results. MRI imaging was from various centers which should be limited to a single type of machine. As both single-voxel and multi-voxel techniques were used, the possibility of sampling errors in tumors of heterogeneous appearance cannot be excluded.Chance of contamination of 1H-MRS voxel by normal brain tissue as 1H-MRS taken by different person in MRI room.Tissue specimen may not be taken from that region from where voxel was taken. So there may be chance of incorrect result by 1H-MRS as glial tumor shows differentiation within the tumor so one tumor may be present in different grade.

Conclusion:

This study have demonstrated that the Myo-inositol/ Creatine ratio has significant correlation with grade of glioma with correlation coefficient value of r = -0.611with a significant p-value of p = <0.001. This can help a clinician to predict the outcome of the patient before any intervention.To make it more sensitive and specific a multicentric trial with larger sample size should be done.

Conflict of interest

The authors declare that there is no conflict of interest.

References:

1. Aydýn, H., Sipahioglu, S., Oktay, N.A., Altýn, E., Kýzýlgöz, V. and Hekimoglu, B. 2011, 'The value of proton mr-spectroscopy in the differentiation of brain tumours from non-neoplastic brain lesions', Journal Belge de Radiologie - Belgisch Tijdschrift voor Radiologi, vol.94, no.1, pp.1–10.
2. Barker, F.G., Chang, S.M., Huhn, S.L., Davis, R.L., Gutin, P.H., McDermott, M.W., Wilson, C.B. and Prados, M.D. 1997, 'Age and the risk of anaplasia in magnetic resonance nonenhancing supratentorial cerebral tumors', International Journal of the American Cancer Society, vol.80, no.5, pp.936-941.

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3. Bertholdo, D., Watcharakorn, A. and Castillo, M. 2013, ‘Brain proton magnetic resonance spectroscopy: introduction and overview’, Neuroimaging Clinics, vol.23, no.3, pp.359-380.

4. Brand, A., Landsberg, C.R., Leibfritz, D. 1993, ‘Multinuclear NMR studies on the energy metabolism of glial and neuronal cells’, Developmental Neuroscience, vlo.15, no.1, pp.289-298.

5. Bulik, M., Jancalek, R., Vanicek, J., Skoch, A. and Mechl, M. 2013, ‘Potential of MR spectroscopy for assessment of glioma grading’, Clinical Neurology and Neurosurgery, vol.115, no.2, pp.146-153.

6. Castillo, M., Smith, J. K. and Kwock, L. 2000, ‘Correlation of myo-inositol levels and grading of cerebral astrocytomas’ American Journal of Neuroradiology, vol.21, no.10, pp.1645-1649.

7. Chawla, S., Wang, S., Wolf, R.L., Woo, J.H., Wang, J., O’Rourke, D.M., Judy, K.D., Grady, M.S., Melhem, E.R. and Poptani, H. 2007, ‘Arterial spin-labeling and MR spectroscopy in the differentiation of gliomas’, American Journal of Neuroradiology, vol.28, no.9, pp.1683-1689.

8. Daumas Duport, C., Scheithauer, B., O’Fallon, J. and Kelly, P. 1988, ‘Grading of astrocytomas: a simple and reproducible method’, Cancer, vol.62, no.10, pp.2152-2165.

9. Essig, M., Anzalone, N., Combs, S.E.,Dorfer, A., Lee, S.K., Picozzi, P., Rovira, A., Weller, M., Law, M. 2012, ‘MR imaging of neoplastic central nervous system lesions: Review and recommendations for current practice’, American Journal of Neuroradiology, vol.33, no.5,pp. 803–817.

10. Fougere, C.L., Suchorska, B., Bartenstein, P., Kreth, F.W. and Tonn, J.C. 2011, ‘Molecular imaging of gliomas with PET: opportunities and limitations’, Neuro-oncology, vol.13, no.8, pp.806-819.

11. Ganau, L., Paris, M., Ligarotti, K., Ganau, M. 2015, ‘Management of gliomas: overview of the latest technological advancements and related behavioral drawbacks’, Behavioural Neurology, vol.2015, no.1, pp.1-7.

12. Gladson, C. L., Prayson, R. A. and Liu, W. M. 2010 ‘The pathobiology of glioma tumors’, Annual Review of Pathology: Mechanisms of Disease, vol.5, pp. 33–50.

13. Hall, W.A., Martin, A., Liu, H. and Truwit, C.L. 2001, ‘Improving diagnostic yield in brain biopsy: coupling spectroscopic targeting with real-time needle placement’, Journal of Magnetic Resonance Imaging, vol.13, no.1, pp.12-15.

14. Hattingen, E., Raab, P., Franz. K., Zanella, F.E., Lanfermann, H., Pilatus, U. 2008, ‘Myo inositol: a marker of reactive astrogliosis in glial tumors’, NMR in Biomedicine: An International Journal Devoted to the Development and Application of Magnetic Resonance In vivo, vol.21, no.3, pp.233-241.

15. Horská, A. and Barker, P.B. 2010, ‘Imaging of brain tumors: MR spectroscopy and metabolic imaging’, Neuroimaging Clinics, vol.20, no.3, pp.293-310.

16. Howe, F.A., Barton, S.J., Cudlip, S.A., Stubbs, M., Saunders. D.E., Murphy, M., Wilkins, P., Opstad, K.S., Doyle, V.L., McLean, M.A., Bell, B.A., Griffiths, J.R. 2003, ‘Metabolic profiles of human brain tumors using quantitative in vivo 1H magnetic resonance spectroscopy’, Magnetic Resonance in Medicine, vol.49, no.2, pp.223-232.

17. Isaacks, R.E., Bender, A.S., Kim, C.Y., Prieto, N.M. and Norenberg, M.D. 1994 ‘Osmotic regulation of myo-inositol uptake in primary astrocyte cultures’, Neurochemical research, vol.19, no.3, pp.331-338.

18. Jackson, R.J., Fuller, G.N., Abi-Said, D., Lang, F.F., Gokaslan, Z.L., Shi, W.M., Wildrick, D.M. and Sawaya, R. 2001, ‘Limitations of stereotactic biopsy in the initial management of gliomas’, Neuro-oncology, vol.3, no.3, pp.193-200.

19. Kim, J.H., Chang, K.H., Na, D.G., Song, I.C, Kwon, B.J., Han, M.H and Kim, K. 2006, ‘3T 1H-MR spectroscopy in grading of cerebral gliomas: comparison of short and intermediate echo time sequences’, American Journal of Neuroradiology, vol.27, no.7, pp.1412-8.

20. Kousi, E., Tsougos, I. and Eftychi, K. 2013, ‘Proton Magnetic Resonance Spectroscopy of the Central Nervous System’, Novel Frontiers of Advanced Neuroimaging. InTech., pp.23-48,doi: 10.5772/53892.

21. Laino M.E., Young, R., Beal, K., Haque, S., Mazaheri, Y., Corrias, G., Bitencourt, A.G., Karimi, S., Thakur, S.B., 2020, ‘Magnetic resonance spectroscopic imaging in gliomas: clinical diagnosis and radiotherapy planning’, British Journal of Radiology, vol.2, no.1,pp.1-12.

22. Law, M., Yang, S., Wang, H., Babb, J.S., Johnson, G., Cha, S., Knopp, E.A. and Zagzag, D. 2003, ‘Glioma grading: sensitivity, specificity, and predictive values of perfusion MR imaging and proton MR spectroscopic imaging compared with conventional MR imaging’, American Journal of Neuroradiology, vol.24, no.10, pp.1989-98.

23. Liang, J., Xiaomin, L., Changyu, L., Xun, Y., Xiaolin, C., Jia, F., Chenghua, L. and Yuanli, Z. 2020, ‘Prognostic factors of patients with Gliomas- A n analysis on 335 patients with Glioblastoma and other forms of Gliomas’, BMC Cancer, vol.20, no.1, pp.1-7.

24. Louis, D.N., Ohgaki, H., Wiestler, O.D., Cavenee, W.K., Burger, P.C., Jouvet, A., Scheithauer, B.W.and Kleihues, P. 2007, ‘The 2007 WHO classification of tumours of the central nervous system’, Acta Neuropathol, vol.114, no.2, pp.97-109.

25. Louis, D.N., Perry, A., Reifenberger, G., Deimling, A., Figarella-Branger, D., Cavenee, W.K., Ohgaki, H., Wiestler, O.D., Kleihues, P. and Ellison, D.W., 2016, ‘The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary’, Acta Neuropathol, vol.131, no.6, pp.803-820.

26. Lu, H., Pollack, E., Young, R., Babb, J.S., Johnson. G., Zagzag, D., Carson, R., Jensen, J.H., Helpern, J.A., Law, M., ‘Predicting grade of, cerebral glioma using vascular-space occupancy MR imaging’, American Journal of Neuroradiology, vol.29, no.2, pp.373-378.

27. Majós, C., Julià-Sapé, M., Alonso, J., Serrallonga, M., Aguilera, C., Acebes, J.J., Arús, C. and Gili, J. 2004, 'Brain tumor classification by proton MR spectroscopy: comparison of diagnostic accuracy at short and long TE', American Journal of Neuroradiology, vol.25, no.10, pp.1696-1704.

28. Metwally, L.I., El-din, S.E., Abdelaziz, O., Hamdy, I.M., Elsamman, A.K. and Abdelalim, A.M. 2014, 'Predicting grade of cerebral gliomas using Myo-inositol/Creatine ratio', The Egyptian Journal of Radiology and Nuclear Medicine, vol.45, no.1, pp.211-217.

29. Mikkelsen, V. E., Solheim, O., Salvesen, O. and Torp, S.H. 2020, 'The histological representativeness of glioblastoma tissue samples', Acta Neurochirurgica, doi: 10.1007/s00701-020-04608-y.

30. Mullins, M. E. 2006, 'MR spectroscopy: truly molecular imaging; past, present and future', Neuroimaging Clinics of North America, vol.16, no.4, pp. 605–618.

31. Nuño, M.,Birch, K., Mukherje, D., Sarmiento, M., Black, K.L. and Patil, C.G. 2013, 'Survival and prognostic factors of anaplastic gliomas', Congress of Neurological Surgeon, vol.73, no.3, pp. 458–465.

32. Ohgaki, H., Kim, Y. H. and Steinbach, J. P. 2010, 'Nervous system tumors associated with familial tumor syndromes', Current Opinion in Neurology, vol.23, pp. 583–591.

33. Ostrom, Q.T., Gittleman, H., Liao, P., Rouse, C., Chen, Y., Dowling, J., Wolinsky, Y., Kruchko, C. and Barnholtz-Sloan, J. 2014, 'CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011', Neuro Oncology, vol.16, no.4,pp.1-63.

34. Parkin, D.M., Bray, F.I. and Devesa, S.S. 2001, 'Cancer burden in the year 2000. The global picture', European Journal of Cancer, vol.37, no.8, pp.4-66.

35. Pathology, R. S. (no date) Limitations of stereotactic biopsy in the initial management of gliomas 1,2, Neuro-Oncology.

36. Rasmussen, B.K., Hansen, S., Laursen, R.J., Kosteljanetz, M., Schultz, H., Nørgård, B.M., Guldberg, R. and Gradel, K.O. 2017, 'Epidemiology of glioma: clinical characteristics, symptoms, and predictors of glioma patients grade I-IV in the the Danish Neuro-Oncology', Journal of Neurooncology, vol.135, no.3, pp.571-579.

37. Rees, J., Watt, H., Jager, H.R., Benton, C., Tozer, D. Tofts, P. and Waldman, A. 2009, 'Volumes and growth rates of untreated adult low-grade gliomas indicate risk of early malignant transformation', European Journal of Radiology, vol.72, no.1, pp. 54–64.

38. Schwartzbaum, J.A., Fisher, J.L., Aldape, K.D. and Wrensch, M. 2006, 'Epidemiology and molecular pathology of glioma', Nature Clinical Practice Neurology, vol.2, no.9, pp.494-503.

39. Sihn, G., Walter, T., Klein, J.C., Queguiner, I., Iwao, H., Nicolau, C., Lehn, J.M., Corvol, P. and Gasc, J.M. 2007, 'Anti-angiogenic properties of myo-inositol trispyrophosphate in ovo and growth reduction of implanted glioma', Federation of European Biomedical Societies, vol.581, no.5, pp.962–966.

40. Sim, H. W., Morgan, E. R. and Mason, W. P. 2018, 'Contemporary management of high-grade gliomas', CNS Oncology, vol.7, no.1, pp. 51–65.

41. Smith, J.K., Castillo, M. and Kwock, L. 2003 'MR spectroscopy of brain tumors', Magnetic Resonance Imaging Clinics, vol.11, no.3, pp.415-429.

42. Soares, D. P. and Law, M. 2009, 'Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications', Clinical Radiology, vol.64, pp. 12–21.

43. Taylor, L.P. 2010, 'Diagnosis, treatment, and prognosis of glioma: five new things' Neurology, vol.75, no.18, pp.28-32.

44. Toh, C.H., Castillo, M., Wei, K.C. and Chen, P.Y. 2020, 'MRS as an aid to diagnose malignant transformation in low-grade gliomas with increasing contrast enhancement', American Journal of Neuroradiology, vol.41, no.9, pp.1592-1598.

45. Verma, A., Kumar, I., Verma, N., Aggarwal, P. and Ojha, R. 2016, 'Magnetic resonance spectroscopy - Revisiting the biochemical and molecular milieu of brain tumors', BBA Clinical, vol.5. pp. 170–178.

46. Wang, J., Hu, G. and Quan, X. 2019, 'Analysis of the factors affecting the prognosis of glioma patients', Open Medicine (Poland), vol.14, no.1, pp. 331–335.

47. Wang, Q., Zhang, H., Zhang, J., Wu, C., Zhu, W., Li, F.Y., Chen, X. and Xu, B. 2016, 'The diagnostic performance of magnetic resonance spectroscopy in differentiating high-from low-grade gliomas: A systematic review and meta-analysis', European Radiology, vol.26, no.8, pp. 2670–2684.

48. Weller. M., Wick, W., Aldape, K., Brada, M., Berger, M., Pfister, S.M., Nishikawa, R., Rosenthal, M., Wen, P.Y., Stupp, R. and Reifenberger, G. 2015, 'Glioma', Nature Reviews, vol.16, no.1, pp.1-18

49. Winn, R.H. (6^{th} eds.) 2011. Youmans neurological surgery, 4 vollume set, Elsivier Saunders, Philadelphia.

50. Wyss, M., and Kaddurah-Daouk, R. 2000, 'Creatine and creatinine metabolism', Physiological Reviews, vol.80, no.3, pp.1107-213.

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