ORIGINAL ARTICLE

Immunoexpression of Ki-67 and p53 in Different Grades of Astrocytoma

Mohammad Rabiul Haque[1], Md. Zahid Hossain[2], Alimul Hasan[3],
Md. Fakhrul Hassan[4], Khadiza Khanam[5]

DOI : https://doi.org/10.47648/jmsr.2023.v3502.01

Abstract:

Astrocytomas are the most common intrinsic tumors of the central nervous system and derive from astrocytes. Ki-67 and p53 are two cellular proteins that have roles in pathogenesis and evolution of astrocytomas. Ki-67 is a nuclear non-histone protein produced by cells in the proliferative phases, and the Ki-67 proliferative index quantitatively reflects the growth potential of tumors, making it a significant biological marker. p53 is a nuclear protein that controls cell cycle, apoptosis, and genomic stability. Assessment of Ki-67 and p53 in various grades of astrocytoma can aid as an ancillary tool in understanding tumor behavior. This cross-sectional type of descriptive study was carried out at the Department of Pathology, RMC, Rajshahi, and BSMMU, Dhaka, over two years, between January 2021 and December 2022. In this study, a total of 30 astrocytoma cases that were histologically diagnosed were included. Ki-67 and p53 immunostaining was done on formalin-fixed paraffin-embedded tissue blocks of all cases and compared with WHO histological grading of astrocytomas. Both biomarkers were directly associated with each other and the grade of astrocytoma. Statistically significant differences in Ki-67 LI and p53 LI were seen between grade I and grade IV and between grade II and IV. However, there was no statistically significant difference between grades I and II, grades I and III, or grades II and III. These markers can aid as an ancillary tool in understanding tumor behavior and progression.

Key words: Astrocytoma, Ki-67, p53

1. Assistant Professor, Department of Pathology, Holy Family Red Crescent Medical College, Dhaka, Bangladesh
2. Clinical Pathologist, Department of Pathology, Rajshahi Medical College Hospital, Rajshahi, Bangladesh
3. Clinical Pathologist, Department of Pathology, Rangpur Medical College Hospital, Rangpur, Bangladesh
4. Lecturer, Department of Pathology, Kushtia Medical College, Kushtia, Bangladesh
5. Professor & Head, Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh

Corresponding Author: rabiul.haque@gmail.com

Introduction:

Astrocytomas are the most common intrinsic tumors of the central nervous system.[1] Annually, intracranial tumors occur in 10 to 17 out of every 100,000 people, while intraspinal tumors are reported in 1 to 2 per 100,000 individuals.[2] Astrocytomas form the largest group of gliomas (>75%), and diffusely infiltrating astrocytomas comprise more than 60% of all the primary brain tumors.[3] The histopathological examination still serves as the gold standard for diagnosing astrocytoma, and histopathological grading is well accepted for assessing the prognosis of patients with astrocytomas. Several histopathological grading systems are used to grade astrocytomas. The WHO classification, the standard system for grading astrocytomas, relies on criteria such as cellular atypia, mitotic rate, vascular changes, and necrosis, dividing them into four grades: I (pilocytic astrocytoma), II (diffuse astrocytoma), III (anaplastic astrocytoma), and IV (glioblastoma).[1] However, the morphologic criteria are not always accurate prognostic indicators. Histological differentiation may not be apparent in some cases, mainly when only small fragments

of tissue biopsies are available. Hence, there is a need to provide an ancillary tool to understand tumor behavior in astrocytoma. Numerous studies have utilized various factors, ranging from tumor suppressor genes to proliferation markers, to gain insights into tumor behavior. Ki-67 and p53 are two cellular proteins that have roles in the pathogenesis and progression of astrocytoma. Ki-67 is a nuclear non-histone protein antigen expressed as 320 and 359 kDa isoforms derived from differentially spliced mRNA variants encoded by the human MKI67 gene.[4] Its presence is noted throughout the G1, S, G2, and M phases, while it is absent in cells in the quiescent G0 phase.

The monoclonal antibody MIB-1 detects Ki-67 nuclear antigens in proliferating cells, and the percentage of immunopositive cells is referred to as the Ki-67 labeling index (LI). The Ki-67 labeling index (proliferative index) estimates the growth of neoplasms quantitatively and can act as an ancillary tool in understanding tumor behavior. The product of the normal TP53 gene is a nuclear phosphoprotein known as p53 ("wild type" p53 protein). It is one of the major factors governing cell proliferation, suppressing growth, and cell transformation. "Wild type" p53 protein has a shorter half-life and is present in small amounts within the normal cell nucleus. Therefore, it cannot be detected by routine immunohistochemical methods. Mutations in the TP53 gene, which modulates anti-proliferative cellular responses, are the most frequent changes in high-grade astrocytomas.[5] Mutations result in the alteration of the p53 protein, and this "mutant" protein, metabolically more stable with a longer half-life, accumulates in the nucleus, reaching a threshold of immunohistochemical detection.[6] There were few studies regarding the immunoexpression of Ki-67 and p53 in astrocytoma in our country. This study aimed to assess the immunoexpression of Ki-67 and p53 in histopathologically diagnosed astrocytomas of different grades.

Methodology:

This descriptive cross-sectional study took place in the Pathology Department of Rajshahi Medical College (RMC) between January 2021 and December 2022, covering 24 months. It included 30 patients who were histopathologically diagnosed as having astrocytoma. Histopathological examination was done in the Department of Pathology, RMC. Immunoexpression of Ki-67 and p53 were evaluated in 30 formalin-fixed and paraffin-embedded blocks of astrocytoma in Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The Ki-67 Labeling Index and p53 Labeling Index were calculated as a percentage of labeled nuclei per 1000 cells. Using a high-power light microscope, 1,000 tumor cells were counted in several tissue areas with uniform positive nuclear staining. When staining was uneven, cells were instead counted in regions visually identified as having the highest density of positive nuclei.

Later, results were recorded, and the data was analyzed using the Statistical Program for Scientific Study (SPSS) software, version 29.0.0. Descriptive analytical techniques involving frequency distribution, computation of percentage, mean, SD, etc., were applied. Fisher's exact test evaluated significant differences between non-parametric variables.

Inclusion and exclusion criteria:

All cases of astrocytoma diagnosed by histopathological examination were included in this study. Patients with recurrent cases and patients who have taken neo-adjuvant or adjuvant chemotherapy and radiotherapy were excluded from this study.

Results:

Distribution of Patients According to Age:
The study sample's age ranged from 3 to 69 years. The majority (26.7%) of the patients belonged to the 21to 30-year-old age group. The next most frequent age group was 51to 60-year-olds (23.3%). The mean age (±SD) was 35.76 ± 23.03 years.

Distribution of Patients According to Gender:
In this study 18 cases (60%) were female, 12 cases (40%) were males and male-to-female ratio was 1:1.5.

Distribution of the Patients According to Astrocytoma Grade:

In this study Grade II was the predominant grade (53.33 %), followed by grade IV (23.33 %). Grade III was the least common among all four grades (10 %).

Table 1: Histopathological Findings in Different Grades of Astrocytoma

Histo-pathological criteria	Nuclear pleomorphism			Mitosis / HPF (Range)	Vascular proliferation		Necrosis	
	mild	moderate	severe		present	absent	present	absent
Grade I	4 (100%)	0 (0%)	0 (0%)	0	0 (0%)	4 (100%)	0 (0%)	4 (100%)
Grade II	16 (100%)	0 (0%)	0 (0%)	0	0 (0%)	16 (100%)	0 (0%)	16 (100%)
Grade III	0 (0%)	2 (66.66%)	1 (33.33%)	3-8	3 (100%)	0 (0%)	0 (0%)	3 (100%)
Grade IV	0 (0%)	0 (0%)	7 (100%)	6-8	7 (100%)	0 (0%)	6 (85.71%)	1 (14.28%)

Mean Ki-67 Labeling Index in Different Grades of Astrocytoma:

The mean Ki-67 Labeling Index gradually increased from histopathological Grade I (3.75) to Grade IV (40.1429). Mean Ki-67 LI for grade II and III were 6.6250 and 23.6667 respectively.

Mean p53 Labeling Index in Different Grades of Astrocytoma:

The mean of p53 Labeling Index gradually increased from histopathological Grade I (4.25) to Grade IV (45.8571). Mean p53 Labeling index for grade II and III were 4.4375 and 20 respectively.

Table 2: Association of Ki-67 LI With Histopathological Grade of Astrocytoma

Grade	Low Ki-67 LI (<10%)	High Ki-67 LI (>10%)	Total	P Value
I	4 (100%)	0 (0%)	4	
II	14 (87.5%)	2 (12.5%)	16	
III	1 (33.33%)	2 (66.66%)	3	<0.001
IV	1 (14.28%)	6 (85.71%)	7	
Total	20 (66.66%)	10 (33.33%)	30	

Data were analyzed using Fisher's exact test; P value <0.001

Table 2 showed significant direct association between grade and Ki-67 LI with P value <0.001. Low Ki-67 LI was predominant in grade I and II, on the other hand, high Ki-67 LI score was more frequent in grade IV and III than other grades.

Table 3: Associationof p53 LIwith Histopathological Grade of Astrocytoma

Grade	Low p53 LI (<20%)	High p53 LI (>20%)	Total	P Value
I	4 (100%)	0 (0%)	4	
II	15 (93.75%)	1 (6.25%)	16	
III	1 (33.33%)	2 (66.66%)	3	<0.001
IV	1 (14.28%)	6 (85.71%)	7	
Total	20 (66.66%)	09	30	

Data were analyzed using Fisher's exact test; P value <0.001

Table 3 showed significant direct association between grade of astrocytoma and p53 LI with P value <0.001. Low p53 LI was predominant in grade I and II, on the other hand, high p53 LI was more frequent in grade III and grade IV.

Table 4: Association of Ki-67 LI With p53 LI

	Low p53 LI (<20%)	High p53 LI (>20%)	Total	P Value
Low Ki-67 LI (<10%)	20	0	20	
High Ki-67 LI (>10%)	1	9	10	<0.001
Total	21	9	30	

Data were analyzed using Fisher's exact test; P value <0.001

Table 4 showed significant direct association between Ki-67 LI and p53 LI with P value <0.001.

P Value on Association of Ki-67 LI Between Different Grades of Astrocytomas:

Statistically significant differences in Ki-67 LI between grade I and grade IV (p=0.015), and between grade II and IV (p=0.002) were seen in this study. However, there was no statistically significant difference between grade I and II (p=1), grade I and III (p=0.143), grade II and grade III (p=0.097) or between grade III and IV (p=1). Data were analyzed using Fisher's exact test.

P Value on Association of p53 LI Between Different Grades of Astrocytomas:

Statistically significant differences in p53 LI between grade I and grade IV (p=0.015), and between grade II and IV (p<0.001) were seen in this study. However, there was no statistically significant difference between grade I and II (p=1), grade I and III (p=0.143), grade II and grade III (p=0.051) or between grade III and IV (p=1). Data were analyzed using Fisher's exact test.

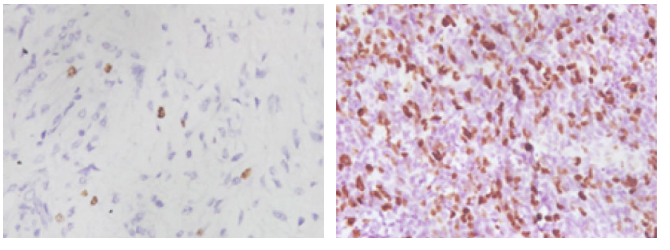

Figure 1: Photomicrograph showing Ki-67 antigen expression in grade I astrocytoma (Case No. T02, x400) and grade IV astrocytoma (Case No. T01, x400) respectively

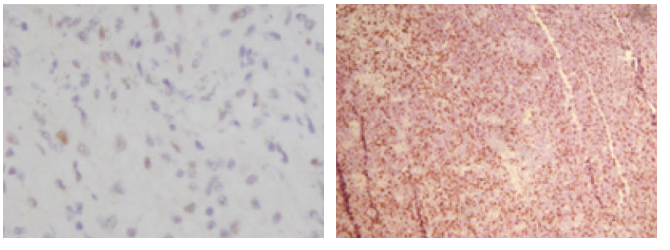

Figure 02: Photomicrograph showing p53 expression in grade Iastrocytoma (Case No. T02, x400) and grade IV astrocytoma (Case No. T01, x200) respectively

Discussion:

In this study, a total of 30 astrocytoma cases that were histologically diagnosed were included. The age ranged from 03 years to 69 years. Overall mean age was 37.6±23.03 years. Similarly, Chaloob *et al.*[7] and Arshad *et al.*[8] showed the mean age of the glial tumor patients was 35.98 years (age range 2-68 years) and 35 years (age range 5 - 67 years) respectively.In our study, it was observed that most patients (26.7%) were in the 3rd decade. The findings were similar to the study by Begum A and Kamal M in 2017, who also found that most patients were in their 3rd decade (31%).[9] Among the thirty specimens, 4 (13.3 %) were histopathologically grade I, 16 (53.3 %) were grade II, 3 (10 %) were grade III, and 7 (23.3 %) were grade IV. Most Pilocytic astrocytoma (grade I) occurred in the first and second decades, which correlated with Giannini *et al.*[10] and Ostrom *et al.*[11] studies. Glioblastoma mainly happened in the 5th to 6th decade, which correlated with the studies of

Thotakura M *et al*.[12] and Katsetos CD *et al*.[13] In the current study, females predominated in grades II, III, and IV. Overall, the male-to-female ratio was 1: 1.5. The slightly higher ratio in females is similar to studies done in Mexico by Anaya-Delgadillo G *et al*.[14] and in the United States by Porter KR et al.[15]. However, in studies done by McKinney *et al*.[16], the male-to-female ratio was 1.2: 1. In a study by Giannini et al.10, the male-to-female ratio was 1.6:1.

In this study, 6 out of the 7 cases of grade IV astrocytoma showed necrosis. Necrosis was absent in grades I, II, and III. All the cases of grade III and grade IV astrocytoma had high mitotic counts. These findings were similar to those of the studies done by Begum A and Kamal M9 in 2017 and Mikkelsen *et al*.[17] in 2021.

The current study gradually increased the mean Ki-67 Labeling Index from Grade I to Grade IV. This result is similar to Thotakura M et al.12 and Das B. *et al*.[18] The findings also match studies performed by Meghdadi and Mahzouni[20] and Qaisrani *et al*.[21] In a similar study by Ambroise *et al*.[19], the mean Ki-67 Labeling Index of Grade I was higher than that of Grade II, while the mean value gradually increased in grades III and IV.High Ki-67 LI was more frequent in grades III and IV compared to tumors in grades I and II. In addition, there was a marked positive association (P<0.001) between the grade of tumor and Ki-67 LI. This result agrees with the studies done by Wakimotoet al.[22] (72 cases), Ambroise *et al*.[19] and Thotakura M *et al*.[12] This finding also matches the studies performed by Sharma *et al*.[23] The findings are nearly equivalent to Singh *et al*.[5], who noted that the Ki-67 labeling index between low-grade & high-grade astrocytoma is statistically significant (P = 0.001). Thus, Ki-67 LI is useful for differentiating between low-grade & high-grade astrocytoma. Still, the differentiation between grade I & grade II, or between grade III & grade IV, is more doubtful due to the overlap of values between various tumor grades. In the current study, there were statistically highly significant differences in Ki-67 LI between grade I and grade IV (P=0.015), grade II and IV (P<0.001). However, there was no statistical difference between grades I and II (P=1), grades II and III (P=0.097), or between grades I and III (P=0.143). Thus, Ki-67 LI might help differentiate between grades I and IV and between grades II and IV. Klein and Roggendorf described that proliferation rates in astrocytomas are seen in the proliferation of tumor and microglial cells, especially in pilocytic astrocytomas[24]. Similar observations were seen in Ambroise *et al*[19].

The mean p53 labeling index gradually increased from Grade I to Grade IV in the current study. This result is similar to those of the studies done by Tihan T et al.[25] and Nayak A *et al*.[26] There was a highly significant association between the histopathological grade of astrocytomas and p53 LI (P<0.001) between low and high grades of astrocytoma. Studies by Sharma *et al*.[23] and Belghali *et al*.[27] also recommended similar findings (P-value < 0.05). In the current study, there were statistically highly significant differences in p53 LI between grade I and grade IV (P=0.015) and between grade II and IV (P<0.001). However, there was no statistical difference between grades I and II (P=1), between grades II and III (P=0.051), or between grades I and III (P=0.143). This means that p53 overexpression was significantly associated with grade IV astrocytoma. This result agreed with Haapasalo *et al*.[28], Pollack *et al*.[29], and Arshad et al.8. However, the current study differed from the findings of Abdelaziz *et al*.[30] in Egypt. Singh et *al*.[5] found no statistically significant association between p53 and astrocytoma grades (P-value 0.07).

Comparison of the Ki-67 labeling index with the p53 labeling index revealed a significant association (p<.001) with each other. This result agreed with the findings of Abdelaziz *et al*.[30] and Mahmood *et al*.[31] Thus, evaluating Ki-67 LI and p53 LI could help differentiate between low-grade & high-grade astrocytoma, particularly between grade I and grade IV and between grade II and grade IV. The findings might also help understand tumor behavior and progression and choose targeted therapy in the future.

Conclusion:

In this study, Ki-67 and p53 labeling indices showed significant differences between grade I and grade IV astrocytoma and between grade II and IV astrocytoma. However, there was no statistically significant difference between grades I and II or between grades I and III. Raised Ki-67 and p53 labeling indices might indicate a more aggressive neoplasm or tumor progressing to a higher grade. These indices might help understand tumor behavior in histologically borderline cases. Patients of low-grade astrocytoma with increased Ki-67 and p53 labeling indices might be at risk of progressing to higher grade. These cases could be detected for close monitoring and follow-up. The findings might also help in choosing targeted therapies and benefit the patient.

References:

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumors of the central nervous system. Acta neuropathologica. 2007 Aug;114:97-109. https://doi.org/10.1007/s00401-007-0243-4

2. Askari K, Janeshin S, Mashouf M, Taherzadeh-Amlashi M, Seyed-Saadat SM. Central nervous system tumors in Guilan, Iran: epidemiological features over 10 years. Caspian Journal of Neurological Sciences. 2015 Mar 10;1(1):19-26. http://dx.doi.org/10.18869/acadpub.cjns.1.1.19

3. Gudinaviciene I, Pranys D, Juozaityte E. Impact of morphology and biology on the prognosis of patients with gliomas. Medicina (Kaunas, Lithuania). 2004 Jan 1;40(2):112-20. https://www.researchgate.net/profile/Inga-Gudinaviciene/publication/7893694_Impact_of_morphology_and_biology_on_the_prognosis_of_patients_with_gliomas/links/0fcfd50dd7a1f5a67a000000/Impact-of-morphology-and-biology-on-the-prognosis-of-patients-with-gliomas.pdf

4. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. Journal of cellular physiology. 2000 Mar;182(3):311-22. https://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-4652(200003)182:3%3C311::AID-JCP1%3E3.0.CO;2-9

5. BHARDWAJ A, SINGH A, KAUSHIK S, KISHORE S, ACHARYA S. Role of Immunohistochemical Markers p53 and Ki-67 in Grading of Glial Tumours: A Prospective Study. Journal of Clinical & Diagnostic Research. 2021 Apr 1;15(4). https://openurl.ebsco.com/EPDB%3Agcd%3A10%3A26193466detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A149997147&crl=c&link_origin=scholar.google.com

6. Nagpal J, Jamoona A, Gulati ND, Mohan A, Braun A, Murali R, Jhanwar-Uniyal M. Revisiting the role of p53 in primary and secondary glioblastomas. Anticancer research. 2006 Nov 1;26(6C):4633-9. https://ar.iiarjournals.org/content/26/6C/4633.short

7. Chaloob MK, Ali HH, Qasim BJ, Mohammed AS. Immunohistochemical expression of Ki-67, PCNA and CD34 in astrocytomas: a clinicopathological study. Oman medical journal. 2012 Sep;27(5):368. https://doi.org/10.5001/omj.2012.93

8. Arshad H, Ahmad Z, Hasan SH. Gliomas: correlation of histologic grade, Ki67 and p53 expression with patient survival. Asian Pac J Cancer Prev. 2010 Jan 1;11(6):1637-40. https://journal.waocp.org/article_25426.html

9. Begum, A. and Kamal, M. Correlation of proliferation index and microvessel density in glial tumors with WHO tumor grades. Journal of Histopathology and Cytopathology. 2017 Jul; 1(2), pp.91-101. https://www.bapath.org/correlation-of-proliferation-index-and-microvessel-density-in-glial-tumors-with-who-tumor-grades/

10. Collins VP, Jones DT, Giannini C. Pilocytic astrocytoma: pathology, molecular mechanisms and markers. Acta neuropathologica. 2015 Jun;129:775-88. https://doi.org/10.1007/s00401-015-1410-7

11. Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C, Barnholtz-Sloan

JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2010–2014. Neuro-oncology. 2017 Oct;19(suppl_5):v1-88. https://doi.org/10.1093/neuonc/nox158

12. Thotakura M, Tirumalasetti N, Krishna R. Role of Ki-67 labeling index as an adjunct to the histopathological diagnosis and grading of astrocytomas. Journal of cancer research and therapeutics. 2014 Jul 1;10(3):641-5. https://doi.org/10.4103/0973-1482.139154

13. Katsetos CD, Dráberová E, Legido A, Dumontet C, Dráber P. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. I. class III β-tubulin. Journal of cellular physiology. 2009 Dec;221(3):505-13. https://doi.org/10.1002/jcp.21870

14. Anaya-Delgadillo G, de Juambelz-Cisneros PP, Fernández-Alvarado B, Pazos-Gómez F, Velasco-Torre A, Revuelta-Gutiérrez R. Prevalence of central nervous system tumours and histological identification in the operated patient: 20 years of experience. Cirugía y Cirujanos (English Edition). 2016 Nov 1;84(6):447-53. https://doi.org/10.1016/j.circen.2016.11.007

15. Porter KR, McCarthy BJ, Freels S, Kim Y, Davis FG. Prevalence estimates for primary brain tumors in the United States by age, gender, behavior, and histology. Neuro-oncology. 2010 Feb 8;12(6):520-7. https://doi.org/10.1093/neuonc/nop066

16. McKinney PA. Central nervous system tumours in children: epidemiology and risk factors. Bioelectromagnetics. 2005;26(S7):S60-8. https://doi.org/10.1002/bem.20149

17. Mikkelsen VE, Solheim O, Salvesen Ø, Torp SH. The histological representativeness of glioblastoma tissue samples. Acta Neurochirurgica. 2021 Jul;163:1911-20. https://doi.org/10.1007/s00701-020-04608-y

18. Das B, Raj KV, Atla B. Clinicohistopathological study of astrocytomas along with Ki-67 proliferative index. Int J Res Med Sci. 2018 Feb;6(6):665-70. http://dx.doi.org/10.18203/2320-6012.ijrms20180317

19. Ambroise MM, Khosla C, Ghosh M, Mallikarjuna VS, Annapurneswari S. Practical value of MIB-1 index in predicting behavior of astrocytomas. Indian Journal of Pathology and Microbiology. 2011 Jul 1;54(3):520-5. https://journals.lww.com/ijpm/fulltext/2011/54030/practical_value_of_mib_1_index_in_predicting.14.aspx

20. Mahzouni P, Mokhtari M, Amirmansour B. Differentiation between reactive gliosis and astrocytomas by MIB-1/ki67 immunostaining. Journal of Research in Medical Sciences. 2007 Sep;12(5):241-5. https://www.sid.ir/EN/VEWSSID/J_pdf/91720070502.pdf

21. Qaisrani AR, Jaffar R, Jahan N, Iqbal T. Comparison of Ki-67/MIB-1 labelling index and histopathological grading of astrocytoma. Pakistan Journal of Physiology. 2017 Jun 30;13(2):19-21. https://www.pjp.pps.org.pk/index.php/PJP/article/view/45

22. Wakimoto H, Aoyagi M, Nakayama T, Nagashima G, Yamamoto S, Tamaki M, Hirakawa K. Prognostic significance of Ki-67 labeling indices obtained using MIB-1 monoclonal antibody in patients with supratentorial astrocytomas. Cancer: Interdisciplinary International Journal of the American Cancer Society. 1996 Jan 15;77(2):373-80. https://doi.org/10.1002/(SICI)1097-0142(19960115)77:2<373::AID-CNCR21>3.0.CO;2-Y

23. Sharma V, Shoaib Y, Gupta L, Dagar A. P53 and Ki-67 expression in primary pediatric brain tumors: Does it correlate with presentation, histological grade, and outcome?. Asian journal of neurosurgery. 2018 Dec;13(04):1026-32. https://www.thieme-connect.com/products/ejournals/abstract/10.4103/ajns.AJNS_69_17

24. Klein R, Roggendorf W. Increased microglia proliferation separates pilocytic astrocytomas from diffuse astrocytomas: a double labeling study. Acta neuropathologica. 2001 Mar;101:245-8. https://doi.org/10.1007/s004010000286 25. Tihan

T, Davis R, Elowitz E, DiCostanzo D, Moll U. Practical Value of Ki-67 and p53 Labeling Indexes in Stereotactic Biopsies of Diffuse andPilocytic Astrocytomas. Archives of pathology & laboratory medicine. 2000 Jan 1;124(1):108-13. https://doi.org/10.5858/2000-124-0108-PVOKAP

26. Nayak A, Ralte AM, Sharma MC, Singh VP, Mahapatra AK, Mehta VS, Sarkar C. p53 protein alterations in adult astrocytic tumors and oligodendrogliomas. Neurology India. 2004 Apr 1;52(2):228-32. https://journals.lww.com/neur/fulltext/2004/52020/p53_protein_alterations_in_adult_astrocytic_tumors.19.aspx

27. Belghali, M., Rais, H., Ba-M'hamed, S., Hazmiri, F., El Khoudri, N., Fakhri, A., Belbachir, A., Elkhiraoui, H. and Hakmaoui, A., 2017. Glial fibrillary acidic protein, CD34, Ki-67, and p53 immunohistochemistry expression study to estimate the concordance between the morphology and the awarded grades of the brain gliomas. Clinical Cancer Investigation Journal, 6(1-2017), pp.44-50. https://doi.org/10.4103/ccij.ccij_148_16

28. Kirla R, Salminen E, Huhtala S, Nuutinen J, Talve L, Haapasalo H, Kalimo H. Prognostic value of the expression of tumor suppressor genes p53, p21, p16 and prb, and Ki-67 labelling in high grade astrocytomas treated with radiotherapy. Journal of neuro-oncology. 2000 Jan;46:71-80. https://link.springer.com/article/10.1023/A:1006473320474

29. Pollack IF, Finkelstein SD, Woods J, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, Boyett JM, Finlay JL, Sposto R. Expression of p53 and prognosis in children with malignant gliomas. New England Journal of Medicine. 2002 Feb 7;346(6):420-7. https://www.nejm.org/doi/full/10.1056/NEJMoa012224

30. Abdelaziz OS, El Sabaa BM, Abdelaziz A. Characterization of Cancer Stem Cells in Patients With Brain Astrocytomas: A Clinicopathologic and Immunohistochemical Study. Neurosurgery Quarterly. 2011 Aug 1;21(3):219-25. https://journals.lww.com/neurosurgery-quarterly/abstract/2011/08000/characterization_of_cancer_stem_cells_in_patients.15.aspx

31. Mahmood MS, Al K, Khafaji K. Expression of Ki 67 and P53 immunohistochemical markers in central nervous system astrocytoma. J Fac Med Baghdad. 2014 Jun; 56 (4): 376-9. https://www.iraqoaj.net/iasj/download/0b11c5b77a84d717