ORIGINAL ARTICLE

Semi-quantitative evaluation of brain gliomas in adults: A focus on neuropathological characteristics

*Marco Antonio Rodríguez-Florido,[1] Iris A. Feria-Romero,[1] Bárbara Nettel-Rueda,[2] José Guerrero-Cantera,[1] Sandra Orozco-Suárez,[1] Jesús A. Chavez,[1] Gerardo Guinto[2], and Israel Grijalva[1]**

[1]Unit of Medical Research in Neurological Diseases; [2]Specialty Hospital, Department of Neurosurgery. Instituto Mexicano del Seguro Social, Centro Médico Nacional Siglo XXI, Mexico City, Mexico

Abstract

Introduction: *Gliomas are neoplasms with high recurrence and mortality. Due to the difficulty to apply the World Health Organization (2016) classification, developing countries continue to use histological evaluation to diagnose and classify these neoplasms.* **Objective:** *To develop a semi-quantitative scale to numerically grade gliomas by its morphological characteristics.* **Method:** *A cohort of patients with gliomas was assessed and followed for 36 months. Tumor tissue sections were analyzed and graded, including aspects such as cell line, cellularity, nuclear pleomorphism, mitosis, endothelial hyperplasia, hypoxic changes, apoptotic bodies, necrosis, hemorrhage and proliferation index.* **Results:** *58 cases were analyzed. Low-grade gliomas median score was 12 points (9 and 13.5 for percentiles 25 and 75, respectively), whereas for high-grade gliomas it was 17 points (16 and 20.5 for percentiles 25 and 75, respectively) ($p < 0.0001$). Thirty-six-month survival of patients with low (13/17) and high grade gliomas (6/41) was also significantly different ($p < 0.0001$).* **Conclusions:** *The semi-quantitative morphological scale allows an objective evaluation of gliomas, with an adequate correlation between the score, tumor grade and survival time.*

KEY WORDS: *Glioblastoma. Low-grade glioma. Semi-quantitative grading. Neuropathological assessment.*

Introduction

Gliomas are primary brain tumors whose precursor cells show morphological and genetical expression similar to those on glial tissue. Gliomas include astrocytomas, oligodendrogliomas and ependymomas. The updated classification of human gliomas is based on the histological criteria issued by the World Health Organization and specific molecular markers.[1] Grade is assigned from I to IV based on morphological characteristics such as vascular proliferation, mitosis, pleomorphism and necrosis, among others. However, these criteria leave room for subjective interpretation and rises intra- and inter-observer variability among neuropathologist. Glioma flowed classification on initial histological evaluation hinders an accurate determination of its incidence and prevalence and can have a negative impact on patient care.[2]

Currently, molecular markers are part of the diagnosis criteria, since the World Health Organization recent classification, in the section of tumors of the central nervous system, includes 1p/19q (LOH) co-deletion, mutation of the IDH1 protein and *ATRX* gene promoter mutation, as part of the mandatory evaluation of gliomas, especially in health care centers where the oligoastrocytomas diagnosis is frequent.[1] Unfortunately, new genotyping techniques or immunohistochemical tests are not always available in developing countries; for this reason, the updated classification has included the "non-specific designation" category when there is

Correspondence:
*Israel Grijalva
E-mail: igrijalvao@yahoo.com

Date of reception: 09-11-2018
Date of acceptance: 19-03-2019
DOI: 10.24875/GMM.M20000329

Gac Med Mex. 2019;155:439-446
Contents available at PubMed
www.gacetamedicademexico.com

no adequate tools available for the description of the cell line.[1] Therefore, with recognition of these limitations, it is important to improve morphological criteria that help determine histological grade, size, localization and cell differentiation patterns, which in addition to improving diagnosis accuracy, contributes prognosis to be established.[3]

In the presented research, the use of a semi-quantitative scale based on morphological characteristics was proposed, including others not currently used for histological classification such as endothelial hyperplasia, apoptotic bodies, hemorrhage and hypoxic changes; this tool is intended to developed a better inter-observer variability, diagnosis and classification of these tumors and serve as a first step for prognosis.

Method

A cohort of patients with presumptive diagnosis of glioma, being taken care at the Department of Neurosurgery of the Specialty Hospital, National Medical Center *Siglo XXI*, who were recruited between 2011 and 2014 and in writing agreed to participate, after receiving information on the research. The present protocol was approved by the National Committee of Scientific Research of the Mexican Institute of Social Security. Inclusion criteria comprised cases with initial presumptive diagnosis of glioma, confirmed by two expert neuropathologists. If a diagnosis mismatch situation ocurred, the assessment of a third neuropathologist was requested. When two neuropathological diagnoses matched, final diagnosis was established. To determine survival time, all patients were followed for at least 36 months after surgery.

Tissue samples, fixed in formalin and embedded in paraffin, were obtained from the hospital's Pathology Department files. Tumor morphology was determined in 5-µm tissue sections, stained with hematoxylin and eosin. Ki67 immunohistochemical detection was carried out with the DIVA™ reagent, Ki67 primary antibody (both from Biocare Medical, Concord, California, USA) and ImmPRESS™ HRP Universal Antibody detection system (anti-mouse IgG/anti-rabbit IgG, peroxidase; Vector Lab, USA). Finally, the secondary antibody was determined with diaminobenzidine (Biocare Medical, Concord, California, USA). All slides were visualized with an optical microscope (model E600, Nikon Eclipse, Japan).

Glioma semi-quantitative assessment

Tumor tissue assessment was carried out with the available slides for each case, depending on tumor size, according to the following characteristics: cell lineage, cellularity, nuclear pleomorphism, mitosis, endothelial hyperplasia, hypoxic changes, apoptotic bodies, necrosis, hemorrhage and proliferation index. According to the cell lineage, tumors were divided into astrocytoma, oligodendroglioma, ependymoma or "non-specific designation", formerly identified as oligoastrocytomas, gangliogliomas or mixed gliomas.[4] Astrocytic neoplasms were assigned a score of 3 (high score), owed to their blurry infiltrative nature and their high recurrence secondary to the difficulty of full resection. "Non-specific designation" neoplasms were assigned a score of 2 (intermediate), due to their mixed astrocytic cell lineage; neoplasms of other cell lineages such as oligodendrogliomas and ependymomas received a score of 1 and 0 (low), respectively, due to their benign behavior (Table 1).

The following variables were assessed according to their characteristics and were assigned a numerical value between 0 and 3: 0 absence, 1 scarce, 2 moderate and 3 abundant (Table 1). Cellularity was determined according to the percentage of cells in relation to their fibrillar mesh (FM, cell: FM) with 10x magnification. Nuclear pleomorphism was assessed based on nuclei size and regularity, as well as on chromatin condensation with a 40x magnification, with a value of 1 being assigned to regular and monotonous nuclei of between 7 and 10 µm in size. The number of mitoses was calculated per field, considering 10 fields with 40x magnification. Endothelial hyperplasia was calculated by the number of vessels per field with 10x magnification. Hypoxic changes were identified by hypereosinophilic neoplastic cells with picnosis, quantifying 10 fields with 40x magnification. Necrosis and hemorrhage were determined by the percentage per field in each sample with 10x magnification. Finally, the proliferation index was determined by the percentage of Ki67 nuclear expression with 40x magnification.

Statistical analysis

The descriptive analysis included frequency, percentages, average ± standard deviation (SD) or median with 25th and 75th percentiles (p25-p75) according to variables' distribution. Differences between histopathological scores of low and high grade tumor groups were estimated using the non-parametric Mann-Whitney U test, and for overall survival, Fisher's exact test was used, calculated with GraphPad Prism version 5.0. A p-value < 0.05 was considered statistically significant.

Table 1. Semi-quantitative morphological assessment of brain gliomas*

Parameter		Scale description and score				Score
Cell lineage	Type Score	Ependymal 0	Oligodendrocyte 1	Non-specific designation 2	Astrocyte 3	
Cellularity Assessment at 10x	Percentage Score	< 10 % 1	11-25 % 2	> 25 % 3		
Nuclear pleomorphism Assessment at 40x	Mild/moderate/ severe Score	Regular and monotonous nuclei, 7-10 μm in size 1	Variable size nuclei of 10-15 μm with compact chromatin 2	Irregular size nuclei >15 μm with granular and open chromatin 3		
Mitosis Ten fields at 40x	Number Score	< 5 1	6-10 2	> 10 3		
Endothelial hyperplasia (blood vessels) Assessment at 10x	Number Score	0 0	1-5 1	6-10 2	> 10 3	
Hypoxia 10 fields at 40x	Number Score	0 0	1-5 1	6-10 2	> 10 3	
Apoptosis Apoptotic bodies Ten fields at 40x	Number Score	0 0	1-5 1	6-10 2	> 10 3	
Necrosis Assessment at 10x	Percentage Score	0% 0	1 to < 30% 1	30 to < 70% 2	> 70% 3	
Hemorrhage Assesment at 10x	Percentage Score	0% 0	1 to < 30% 1	30 to < 70% 2	> 70% 3	
Proliferation index Ki67 nuclear expression Assessment at 40x	Percentage Score	0% 0	1 to < 10% 1	10 to < 20% 2	> 20% 3	
Total score						

*The assessment was carried out in all the slides available for each case. Tissue evaluation at 10x or 40x: the entire case is evaluated with 10x or 40x magnification. Ten fields at 40x: ten different fields are analyzed separately with a 40x magnification. Cellularity is determined by the percentage of cells (cell %) that is observed in relation to their fibrillar mesh (FM). Pleomorphism considers nuclei size and regularity and chromatin condensation. Mitosis quantifies the number of dividing cells per field at 40x. Endothelial hyperplasia is determined by the number of vessels per field. Hypoxic changes are identified by hypereosinophilic neoplastic cells with picnosis. Necrosis and hemorrhage are determined as the percentage in tissue sections per sample. The proliferation index is determined by the percentage of cells with Ki67nuclear expression. Glioma classification: low grade from 3 to 14 points, high grade from 15 to 30 points.

Results

Fifty-eight patients with an average age of 52.1 ± 16.4 years were evaluated; the male gender predominated (60.3 %). The neuropathologists concurred in 48 cases (83 %). A third neuropathologist assessed 10 discordant cases; once the opinion of two evaluators matched, final diagnosis was established. The most common localization for the tumor was in the left hemisphere (56.6 %), with a predominance in the frontal and parietal lobes (38.2 and 28.9 %, respectively); 84 % of the tumors were from astrocytic origin (49/58), with glioblastoma being the most common (65.5 %). The tumors were divided into low (grades I and II) and high grade (grades III and IV). Mean age of patients with low grade gliomas (n = 17) was 44.4 ± 17 years and 55.3 ± 15 years for those with high grade gliomas (n = 41). Male gender predominance was observed in both groups: 65 and 56.1 %, respectively (Table 2).

Morphological characteristics of the tumors included in the semi-quantitative evaluation are shown in Figure 1. The score range of the tumors was 6 to 26 points (including each parameter). The median for low-grade gliomas was 12 points (9-13.5) and 17 points (16-20.5) for high-grade gliomas, which showed a statistically significant difference ($p < 0.0001$).

The scores about main biological characteristics of malignant astrocytomas, such as cell proliferation, angiogenesis and cell death, were estimated. The cell proliferation value was obtained by adding the scores of the following parameters: cellularity, nuclear pleomorphism, mitosis and Ki67; a significant difference was noticed between hign and low grade ($p = 0.0002$). The angiogenesis value was obtained by adding the endothelial hyperplasia and hemorrhage scores, with a

Table 2. Demographics, localization, laterality and diagnosis of patients with low- and high-grade gliomas

Variables	Total			Low-grade			High-grade		
	n	%	Left/right	n	%	Left/right	n	%	Left/right
Demographics									
Age, years ± SD	52.1 ± 16.4			44.4 ± 16.9			55.3 ± 15.2		
Patients	58	100	—	17	29.3	—	41	70.7	—
Males	36	60.3	—	13	65.0	—	23	56.1	—
Affected lobes									
1	35	60.3	18/17	13		8/5	22	53.7	10/12
2	19	32.7	8/11	2	76.5	0/2	17	41.5	8/9
3	1	1.7	0/1	—	11.8	—	1	2.4	0/1
Extralobar	3	5.2	—	2	11.7	—	1	2.4	—
Localization									
Frontal	29	38.2	16/13	10	58.8	6/4	19	32.2	10/9
Parietal	22	28.9	7/15	2	11.8	0/2	20	33.9	7/13
Temporal	16	21.1	5/11	4	23.5	2/2	12	20.3	3/9
Occipital	9	11.8	5/4	1	5.9	0/1	8	13.6	5/3
Histopathological diagnosis									
Glioblastoma	38	65.5	16/22	—	—	—	38	95.0	16/22
A III	1	1.7	0/1	—	—	—	1	2.4	0/1
NSD III	1	1.7	0/1	—	—	—	1	2.4	0/1
E II	1	1.7	1/0	—	—	—	1	2.4	1/0
A II	10	17.2	3/7	10	58.8	3/7	—	—	—
NSD II	5	8.6	4/1	5	29.4	4/1	—	—	—
O II	1	1.7	1/0	1	5.9	1/0	—	—	—
E II	1	1.7	0/1	1	5.9	0/1	—	—	—
Total	58	100	25/33	17	100	8/9	41	100	17/24

A III = grade III anaplastic astrocytoma, NSD III = grade III non-specific designation, E III = grade III anaplastic ependymoma, A II = pleomorphic xanthoastrocytoma, diffuse astrocytoma, gemistocytic astrocytoma; NSD II = grade II non-specific designation, O II = grade II oligodendroglioma, E II = grade II ependymoma.

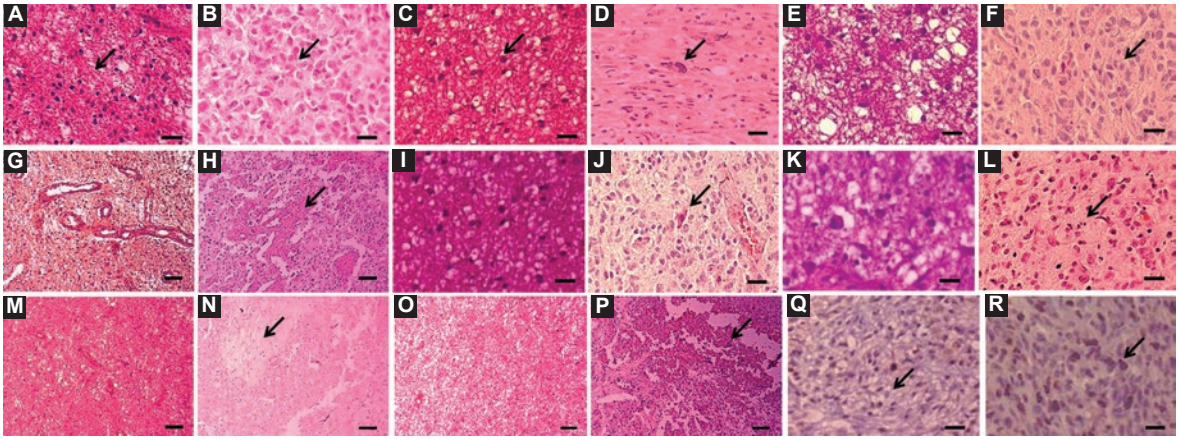

Figure 1. *Cell characteristics for the semi-quantitative morphological evaluation of brain gliomas. The scores correspond to the characteristics indicated in Table 1. Cellularity:* **A:** *score of 2 and cell percentage of 15 %;* **B:** *score of 3 and cell percentage of 50 %. Nuclear pleomorphism:* **C:** *score of 1 (regular nuclei, 7 to 10 µm);* **D:** *score of 3 (irregular nuclei,> 15 µm). Mitosis:* **E:** *score of 1 (0-5 mitoses);* **F:** *score of 3 (> 10 mitoses). Endothelial hyperplasia:* **G:** *score of 0 (absent);* **H:** *score of 3 (> 10 blood vessels). Hypoxia:* **I:** *score of 0 (absent);* **J:** *score of 3 (> 10 cells with picnosis). Apoptosis:* **K:** *score of 0 (absent);* **L;** *score of 3 (> 10 apoptotic bodies). Necrosis:* **M:** *score of 0 (absent);* **N:** *score of 3 (> 70 %). Hemorrhage:* **O:** *score of 0 (absent);* **P:** *score of 3 (> 70 %). Proliferation index:* **Q;** *score of 1 (Ki67 expression <10 %);* **R:** *score of 3 (Ki67 expression > 20 %). The scores were added to obtain a total value and predict the tumor grade. In 40x magnification, the bar corresponds to a 20-µm scale for cellularity, nuclear pleomorphism, mitosis, apoptosis and Ki67 expression. In the 10x magnification, the bar corresponds to a 50-µm scale for endothelial hyperplasia, necrosis and hemorrhage. Histological characteristics are indicated with a black arrow.*

significant difference was found (p = 0.0005). Cell death value was calculated by adding the necrosis and apoptosis scores; a significant difference was also observed (p < 0.0001) (Figure 2).

When the 36-month survival rate was considered, follow-up was only performed in 14/17 patients with low-grade tumor who received treatment (one died in the postoperative period and two were lost during

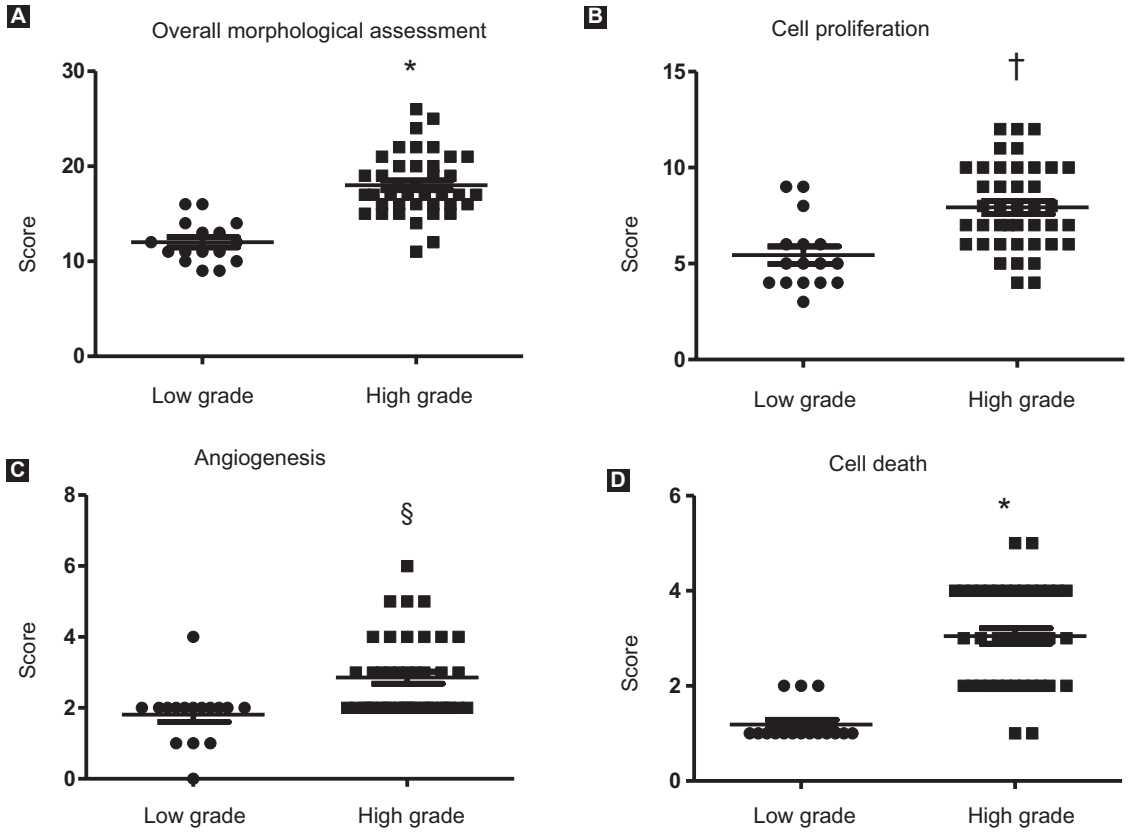

Figure 2. *Differences in low and high grade glioma scores.* **A:** *overall morphological evaluation (includes all parameters), *p < 0.0001.* **B:** *cell proliferation (cellularity + nuclear pleomorphism + mitosis + Ki67), †p < 0.0002.* **C:** *angiogenesis (endothelial hyperplasia + hemorrhage), §p < 0.0005;* **D:** *cell death (necrosis and apoptosis), *p < 0.0001.*

follow-up) and in 22/41 patients with high-grade tumor (five died in the postoperative period, 11 were lost in the follow-up and three refused to postoperative radiotherapy and chemotherapy treatment). For the record, 13/17 patients with low grade (76.4 %) and 6/41 with high grade gliomas (14.6 %) stayed alive at the end of the 36-month follow-up. A considerable gulf was observed between the groups in overall survival ($p < 0.0001$).

Discussion

Due to gliomas heterogeneous characteristics, their classification and tumor staging originates subjective interpretations and discrepancy on diagnosis; therefore, 20 to 30 % of cases are reclassified when at least two neuropathologists review the tumor material.[2] Aldape et al. reported disagreement in the diagnosis in 23 % of 457 evaluated cases –with higher disagreement in the referred cases from community-based hospitals and lower in those from academic hospitals–; they considered that 16 % of discordant diagnoses were clinically relevant for treatment and prognosis.[5] It appears that some inter-observer variations are due to simple technical issues such as not reviewing exactly the same material, but mainly to the use of subjective terms to distinguish tumor grades, for example “cellularity increase” or “moderate cellularity increase”, used to separate anaplastic astrocytoma from a grade II glioma.[4]

Although the combined use of histological and molecular criteria could improve the results,[1] new approaches are required for the classification of tumors owed to the lack of objective, quantitative and reproducible criteria for histological diagnoses. This may be important when considering that molecular techniques are difficult to access in developing countries, where hematoxylin and eosin staining is the first step in diagnosis and the most accessible method for most pathology laboratories.

Therefore, in this work, the sustained aspects of malignant gliomas were selected, which allow for them to be differentiated with higher accuracy from low-grade gliomas through an objective analysis. In

first place, the cell lineage was considered, which can be astrocytic, oligodendrocytic or ependymal, but cases of "non-specific designation" were also identified. The astrocytic lineage is the most common in low to high grade gliomas (I to IV or glioblastoma). The oligodendrocytic lineage is the next one in frequency, which is able to develop up to grade III neoplasms. Finally, the less frequent lineage is ependymal, which may also develop grade III neoplasms.[6] Astrocytic lineage predominated in the analyzed population.

In general, depending on their malignancy, gliomas may submit with necrotic center, vascular proliferation, neoplastic glial cells, mitosis and nuclear pleomorphism, with abundant or insufficient FM. In addition, heterogeneity of structures with epithelial appearance, variations in cell morphology such as small cells, oligoid components, giant cells, gemistocytes, granular cells and lipidized cells, metaplastic components, variable vascular proliferation and coagulative or pseudopalisading necrosis can also be observed, as well as immune response with perivascular lymphocytes. The histopathological aspects of growing tumors are cell: FM ratio, presence of undifferentiated and pleomorphic cells, identification of mitosis or polynucleated cells and Ki67expression. In this work, the percentage or amount of cell proliferation allowed to differentiate between gliomas of low (I and II) and high grade (III and IV).

In high-grade gliomas, neoplastic cells with astrocytic differentiation form thin cytoplasmic extensions with stellar appearance. In contrast, low-grade astrocytomas, such as diffuse astrocytoma, generally show a constant FM, with a higher proportion with regard to the number of cells. The degree of malignancy increases when the number of cells is higher than the FM, as in glioblastoma, so that hypercellular areas can be found where FM is sparse or moderate.[7] Other alterations include differentiated cells, cells with reduced cytoplasm, cells with compact nuclear chromatin or mild to severe nuclear pleomorphism.[8] Glioma cells may be slightly differentiated or spindle-shaped, where high proliferative activity can be observed; however proliferation is low when there is gemistocytic differentiation.[9] This characteristic is associated with the number of mitoses and is a form to determine the degree of tumor malignancy. The number of mitoses is limited in low-grade gliomas and is generally high in glioblastomas, where forms with typical and atypical mitosis can be identified. Cell proliferation, which is determined through Ki67 immunohistochemical detection is comparable among high-grade gliomas, where an expression between 15 and 20 % is observed, which is significantly higher in comparison with low-grade gliomas. The Ki67 protein participates in cell cycle regulation and, therefore, it is absent in cells without replication, and its highest levels of expression are reached during mitosis. In addition, it is commonly used as a marker of cell proliferation and is positively correlated with tumor grade and prognosis.[10]

Angiogenesis is a parameter that encompasses the presence of normal and aberrant vessels, hypoxia and hemorrhage. In the present work, the scores corresponding to angiogenesis and those of cell proliferation were observed to be remarkably different between low- and high-grade gliomas.

Hypoxia and angiogenesis are two related factors and their presence is characteristic in high-grade gliomas. Cell accumulation of hypoxia-induced factor 1 alpha (HIF-1α) activates hypoxia-regulated genes and induces angiogenesis through the expression of vascular endothelial growth factor.[11] In angiogenesis, neoplastic vessels are formed from preexisting vessels through the migration of neoplastic cells. It has also been proposed that neoplastic stem cells regulate and contribute to the formation of neoplastic vessels when they differentiate into endothelial cells or through the secretion of factors such as vascular endothelial growth factor or hepatoma-derived growth factor.[12] Angiogenesis promotes the formation of different microvascular patterns such as glomeruloid tufts, vascular sprouting, vascular clusters, "garlands of vessels", dilated vessels, among others. Initially, the vessels present on the periphery of the lesion can be detected by imaging studies. In the resected tumor, the plenty of vascularization and the presence of endothelial hyperplasia (oval formations that resemble renal corpuscles) are corroborated. Focal endothelial hyperplasia is sufficient to diagnose and elevate the degree of malignancy.[13] In this work it was importantly observed in high-grade gliomas.

As for cell death, glioblastoma main characteristic is the presence of necrosis, because it allows a substancial distinction in low and high grade gliomas average values. In addition, necrosis predicts clinical evolution aggressiveness and is associated with low survival rates.[14] Coagulative necrosis histopathological corroboration in glioma may suggest an insufficient oxygen supply side effect. Another type of characteristic necrosis in glioblastoma is a serpentine-shaped structure with a necrotic center and neoplastic glial cells with a pseudopalisading appearance in the periphery.[15,16] In the center of the tumor, necrotic

tissue or FM remains can be identified, and adjacent to these areas, probably due to central region of hypoxia, apoptotic bodies with low cell proliferation are found.

Another probable cause of necrosis is vascular occlusion by thrombi. This phenomenon occurs in hypoxic areas adjacent to affected vessels and allows cell migration to the periphery of the necrotic area. The percentage of necrosis is variable from one tumor to another, reaching up to 80 % of the neoplastic tissue. Differences in primary and secondary glioblastomas have also been observed, since primary glioblastomas show more necrosis than secondary glioblastomas.[17]

Apoptosis or programmed cell death has distinctive cell morphological characteristics. In this type of cell death, nuclei are distinguished by granular and fragmented chromatin. These changes are initiated through the binding of tumor necrosis factor-related apoptosis inducer ligand receptors, death receptor 5 and caspase 8 subsequent activation. FAS expression has been observed in cells surrounding the pseudopalisade, and FAS-FASL expression, in peripheral cells of the coagulative necrosis area; however, its association with apoptotic processes is still under discussion.[18]

It is important to clarify that, in addition to histopathological factors, clinical aspects such as age, pre-surgical neurological deficit, tumor size and location have also been established; surgical aspects such as grade of resection and biomarkers such as IDH-1mut, MGMT promoter methylation (non-methylated) and 1p19q.3 co-deletion, have also been established as prognostic factors.[19-21] Therefore, the histopathological score, clinical aspects and biomarkers can be used together to predict evolution and establish an aggressive or conservative treatment of glioma.[22,23]

The limitations of this work were determined by the following aspects:

- The small number of patients, hence a sufficient number of gliomas of all grades was not included, which only allowed to differentiate them in low and high grade.
- The limited follow-up of disease evolution, which did not make it possible to determine overall survival in periods longer than three years.

On the other hand, it will be necessary to test the proposed scale in prospective studies that demonstrate that the tool correlates better with tumor progression-free survival and overall survival, which will take time, and require a larger number of patients to support that these criteria are useful. The present work does not include the molecular criteria proposed by the World Health Organization in 2016,[2] since the purpose of this study was to strengthen the assessment of the histological characteristics that can be gauged in developing countries.

It is concluded that the proposed semi-quantitative morphological evaluation allows a more objective histopathological classification. Even when histological grading remains a challenge due to the overlap of morphological characteristics, it can reduce inter-observer diagnosis discrepancies. The proposed tool might allow the pathologist to diagnose and classify gliomas with higher accuracy, which could be used by clinicians as an independent predictor of tumor progression, along with the molecular tests proposed by the World Health Organization, whenever this is possible.

Acknowledgements

We would like to specially thank Dr. Rocío Lorena Arreola Rosales of the Department of Pathological Anatomy, Specialty Hospital, Centro Médico Nacional Siglo XXI, for her valuable help in providing the histopathological material.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

This work was financed by the Health Research Fund of the Mexican Institute of Social Security, FIS/IMSS/PRIO/10/012.

References

1. Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131:803-820. DOI: 10.1007/s00401-016-1545-1
2. Van den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. Acta Neuropathol. 2010;120:297-304.
3. Weller M, Wick W, Aldape K, Brada M, Berger M, Pfister SM, et al. Glioma. Nat Rev Dis Primers. 2015;1:15017.
4. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO Classification of Tumours of the Central Nervous System. Francia: International Agency for Research on Cancer; 2007.
5. Aldape K, Simmons ML, Davis RL, Miike R, Wiencke J, Barger G, et al. Discrepancies in diagnoses of neuroepithelial neoplasms. Cancer. 2000;88:2342-2349.
6. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, et al. CBTRUS Statistical Report: primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. Neuro Oncol. 2015;17:iv1-iv62.
7. Burger PC, Vollmer RT. Histologic factors of prognostic significance in the glioblastoma multiforme. Cancer. 1980;46:1179-1186.

8. Sharma S, Deb P. Intraoperative neurocytology of primary central nervous system neoplasia: a simplified and practical diagnostic approach. J Cytol. 2011;28:147-158.
9. Watanabe K, Tachibana O, Yonekawa Y, Kleiuhes P, Ohgaki H. Role of gemistocytes in astrocytoma progression. Lab Invest. 1997;76:277-284.
10. Thotakura M, Tirumalasetti N, Krishna R. Role of Ki-67 labeling index as an adjunct to the histopathological diagnosis and grading of astrocytomas. J Cancer Res Ther. 2014;10:641-645.
11. Acker T, Plate KH. Hypoxia and hypoxia inducible factors (HIF) as important regulators of tumor physiology. Cancer Treat Res. 2004;117:219-248.
12. Jhaveri N, Chen TC, Hofman FM. Tumor vasculature and glioma stem cells: contributions to glioma progression. Cancer Lett. 2016;380:545-551.
13. Chen L, Lin ZX, Lin GS, Zhou CF, Chen YP, Wang XF, et al. Classification of microvascular patterns via cluster analysis reveals their prognostic significance in glioblastoma. Hum Pathol. 2015;46:120-128.
14. Burger PC, Green SB. Patient age, histologic features, and length of survival in patients with glioblastoma multiforme. Cancer. 1987;59:1617-1625.
15. Brat DJ, Van Meir EG. Vaso-occlusive and prothrombotic mechanisms associated with tumor hypoxia, necrosis, and accelerated growth in glioblastoma. Lab Invest. 2004;84:397-405.
16. Rong Y, Durden DL, Van Meir EG, Brat DJ. "Pseudopalisading" necrosis in glioblastoma: a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. J Neuropathol Exp Neurol. 2006;65:529-539.
17. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clin Cancer Res. 2013;19:764-772.
18. Hao C, Beguinot F, Condorelli G, Trencia A, Van Meir EG, Yong VW, et al. Induction and intracellular regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis in human malignant glioma cells. Cancer Res. 2001;61:1162-1170.
19. Pignatti F, Van den-Bent M, Curran D, Debruyne C, Sylvester R, Therasse P, et al. Prognostic factors for survival in adult patients with cerebral low-grade glioma. J Clin Oncol. 2002;20:2076-2084.
20. Stupp R, Brada M, Van den-Bent MJ, Tonn JC, Pentheroudakis G, ESMO Guidelines Working Group. High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25:iii93-iii101.
21. Adeberg S, Bostel T, König L, Welzel T, Debus J, Combs SE. A comparison of long-term survivors and short-term survivors with glioblastoma, subventricular zone involvement: a predictive factor for survival? Radiat Oncol. 2014;9:95.
22. Towner RA, Jensen RL, Colman H, Vaillant B, Smith N, Casteel R, et al. ELTD1, a potential new biomarker for gliomas. Neurosurgery. 2013;72:77-90.
23. Su J, Guo B, Zhang T, Wang K, Li X, Liang G. Stanniocalcin-1, a new biomarker of glioma progression, is associated with prognosis of patients. Tumour Biol. 2015;36:6333-6339.