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Morphometric study of nuclei and microvessels in gliomas and its correlation with grades

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ABSTRACT

Introduction: Modifications of nuclear morphology in conjunction with alteration in microvascular configuration are essential features encountered during the progression of glial tumors. In order to gain more insight into tumor biology of gliomas, objectives of the study were selected (a) to correlate morphometrically evaluated nuclear parameters [nuclear area (NA), nuclear perimeter (NP), nuclear density (ND), percentage of total nuclear area (%TNA)] and microvessel parameters [microvessel density (MVD), microvessel caliber (VC), microvessel cross sectional area (VCSA), total microvessel boundary density (TVBD), percentage of total VCSA (%TVCSA)] with WHO grading; (b) extend such correlations to the ratio parameters: ratio of MVD to ND (MDV/ND), ratio of TVBD to %TNA (TVBD/%TNA) and ratio of %TVCSA to %TNA (%TVCSA/%TNA); and (c) to correlate microvessel and ratio parameters with NP and ND.

Materials and methods: A total of thirty gliomas managed at this institute during 2009–2012 were evaluated for various nuclear and microvessel parameters by image morphometry using a computerized digital photomicrograph system. For assessment of microvessel parameters CD34-immunostained sections were used while nuclear morphometry was performed on routine hematoxylin-eosin-stained sections. Appropriate statistical analysis was performed in correlation studies.

Results: All nuclear morphometric parameters showed strong positive correlation with tumor grades ($r > 0.7$). In contrast, though all microvessel parameters exhibited positive correlation with grades, the parameters TVBD and %TVCSA showed strong positive correlation. The ratio parameters (MVD/ND) and (TVBD/%TNA) showed negative correlation with grades, whereas (%TVCSA/%TNA) did not exhibit meaningful correlation with grades. Further, while all microvessel parameters showed positive correlation with NP and ND; ratio parameters showed negative correlation with them.

Conclusion: This study indicates that the parameters related to tumor growth (NA, NP, ND, %TNA), and angiogenesis showed increasing trend with tumor grades simultaneously; whereas the parameters related to supply of nutrients per nucleus showed decreasing trends with tumor grades, nuclear size and nuclear density. Thus, the former accounts for increased cellularity, mitosis, and vascular proliferation, while the latter culminates in tumor necrosis, all of which are essential components for grading of gliomas. The present study will therefore have a vital role as surrogate markers of grading of tumor.

Introduction

Gliomas are primary central nerve system (CNS) tumors. They account for half of all primary brain tumors and nearly one-fifth of all primary spinal cord neoplasms (Bondy et al., 2008). Although the exact cause responsible for development of gliomas is unknown, it is presumed that they may have their basis in various genetic and/or environmental factors. Gliomas are characterized by increased mitotic rate, nuclear pleomorphism, high glucose consumption, angiogenesis, intratumoural necrosis and hypoxia, breakdown of blood–brain barrier and brain edema (Aronen et al., 2000).

Currently, it has been well established that several clinical and histopathological parameters are useful in predicting the overall outcome of glioma patients. Presently, well established methods like morphometry, stereology, static image and flow-cytometry are routinely used in diagnostic quantitative pathology. The potential significance of these techniques include the objective distinction between benign, borderline and malignant lesions; objective grading of invasive tumors; prediction of prognosis and therapeutic response. Computer-assisted image morphometry of histopathological sections provides a new powerful tool for high-precision measurement of different aspects of the tumor cells to achieve quantitative information and better perception (Boruah

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and Deb, 2013; Deb et al., 2012; Jalava et al., 2001; Moro-Rodríguez et al., 2008). Further, image morphometry has immense potential for the basic understanding of the tumor process. To date only a few studies have utilized morphometric techniques to determine the mean nuclear size and shape, microvascular profiles and their correlation with tumor grade in neoplastic tissues in CNS tumors (Boruah and Deb, 2013; Bulnes et al., 2009; Deb et al., 2012; Kararizou et al., 2011; Korkolopoulou et al., 2004; Li and Niu, 2010; Moro-Rodríguez et al., 2008; Noy et al., 2011; Pennella et al., 2000; Piperi et al., 2011; Samaras et al., 2009; Serio et al., 1996). To the best of the authors' knowledge no morphometric study has been performed to assess microvessel and nuclear parameters simultaneously in gliomas. Collective study of microvessel and nuclear parameters has the potential to find out the factors related to the supply of nutrients per nucleus, which can give additional input to understand the disease process in CNS tumors. Further, these may also serve as surrogate markers for grading, especially in cases of small biopsies obtained from eloquent areas of the brain and also in cases of stereotactic surgeries.

In the present study the image morphometric technique was used to evaluate various nuclear and microvessel parameters. The parameters evaluated were:

(1) Nuclear parameters: mean nuclear area (NA) depicting nuclear size; mean nuclear perimeter (NP) representing nuclear size and shape; mean nuclear density (ND) and percentage of total nuclear area (% TNA), both of which herald the rate of growth of the tumor;
(2) Microvessel parameters: microvessel density (MVD), microvessel caliber (VC), microvessel cross sectional area (VCSA), total microvessel boundary density (TVBD) and percentage of total VCSA (%TVCSA);
(3) Ratio parameters: ratio of MVD to ND (MDV/ND); ratio of TVBD to %TNA (TVBD/%TNA); and ratio of %TVCSA to %TNA (%TVCSA/%TNA). These ratio parameters reflect the supply of blood and nutrients per nucleus.

The main objectives of this study were: (1) to correlate morphometrically evaluated nuclear parameters (NA, NP, ND, %TNA) and microvessel parameters (MVD, VC, VCSA, TVBD, %TVCSA) with WHO histologic grade of glial tumors; (2) to extend such correlations to the ratio parameters [(MDV/ND), (TVBD/%TNA) and (%TVCSA/%TNA)] in an effort to gain greater insight of the tumor process; and (3) to correlate microvessel and ratio parameters with NP and ND, so as to acquire information about their behaviors with nuclear size and growth rate of the tumor.

Materials and methods

The present study included thirty gliomas managed at this tertiary care neurosurgical institute during 2009–2012. This comprised (a) 14 cases of WHO Grade-II gliomas [ten diffuse astrocytoma, four oligoastrocytoma]; (b) 3 cases of WHO Grade-III gliomas [anaplastic astrocytoma]; and (c) 13 cases of WHO Grade-IV gliomas [GBM: glioblastoma multiforme]. Sections from parts of brain without any pathology were obtained from ten autopsy cases (three died of ischemic stroke; two died consequent to hemorrhagic stroke; and five died of non-CNS causes without any brain pathology). These were divided into four groups: Grade II, Grade III, Grade IV and Control group.

The mean age at surgery was 39.1 years (range: 9–62 years) for Grade-II, 46.7 years (range: 35–65 years) for Grade-III and 56.4 years (range: 27–79 years) for Grade-IV tumors.

Nuclear and vascular morphometry

Nuclear morphometric analysis was performed on H&E stained representative histological sections of 5 micron thickness of formalin fixed paraffin embedded tissue, using a computerized digital photomicrograph system (Boruah and Deb, 2013). For each sample five high power fields (400×) having maximum cellularity, were recorded for the study [Figs. 1(A–D)]. For each case, 100 nuclei which clearly separated from others and relatively larger in size, were chosen to evaluate nuclear shape and size. The nuclei were outlined using a mouse attached to the computer. Then outlined nuclei were separated from others and their nuclear parameters were determined using the software. Nuclei were analyzed for nuclear area (NA) and nuclear perimeter (NP). Counting of nucleus was done in the five recorded high power fields and hence the mean nuclear density (ND) was calculated. The percentage of total nuclear area (%TNA) was determined for the five fields for each sample using the software and their mean was calculated.

Vascular morphometric analysis was performed on CD-34 stained sections from the same tissue block of respective samples chosen for nuclear morphometry [Figs. 1(E–H)]. Five high power fields (400×) in the region having highest vascular density were recorded for each sample. Counting of microvessel has been done in the recorded fields and hence the MVD is calculated. The caliber of each counted microvessel is measured (Boruah et al., 2013; Deb et al., 2012). After measurement, the data has been transferred to MS-Excel sheet for further analysis.

Microvessel parameters: MVD, VC, VCSA, TVBD, %TVCSA and three ratio parameters: (MDV/ND), (TVBD/%TNA) and (%TVCSA/%TNA) were evaluated for each sample. VCSA, TVBD and %TVCSA were calculated using the following relations:

- $VCSA = \{\pi \cdot (VC)^2 / 4\}$.
- $TVBD = \pi \times MVD \times VC$.
- $\%TVCSA = MVD \times VCSA \times 100$.

Statistical analysis

The nuclear parameters NA and NP were analyzed for each nucleus of every sample and their means were determined. Mean ND, %TNA, MVD, VC, VCSA, TVBD, %TVCSA, (MDV/ND), (TVBD/%TNA) and (%TVCSA/%TNA) were determined for each sample. The mean values of these parameters with standard deviation (SD) and range were calculated for the four groups. Student's *t* test was performed to evaluate the difference in all the studied parameters for each pair of the groups and the p-values were determined. Data was reported as mean, standard deviation of mean and range of mean for these parameters. The statistical correlations of the analyzed morphometric parameters with tumor grades of all samples were investigated; correlations of MVD, VC, VCSA, TVBD, %TVCSA, (MDV/ND), (TVBD/%TNA) and (%TVCSA/%TNA) with NP and ND were also studied. Pearson correlation coefficient ('r') and p value were calculated, and regression line was drawn in correlation studies. In this study the control group was designated as Grade 0.

Results

The mean value with SD and range of all studied parameters of the each group (Control, Grade II, Grade III and Grade IV) are presented in the Table 1. The p values for each pair of groups for the studied parameters are presented in Table 2. Figs. 2(A–L) represent the mean values with range of NP, NA, ND, %TNA, MVD, VC, VCSA, TVBD, %TVCSA, (MVD/ND), (TVBD/%TNA) and (%TVCSA/%TNA) of the four groups. Figs. 3(A–H) represent the mean values of MVD, VC, VCSA, TVBD, %TVCSA, (MVD/ND), (TVBD/%TNA) and (%TVCSA/%TNA) with WHO grade (according to WHO classification 2007) with linear regression; where the controls were assigned as Grade 0.

Controls

The mean age of the controls was 46.3 year (range: 40 year–53 year). Nuclei, in the control tissues were evenly distributed, with mean NP of 21.54 μm (range: 18.84 μm–25.33 μm); mean NA was 23.44 μm^2 (range: 19.42 μm^2–30.33 μm^2); ND being 1107 mm^{-2}