Original Article

A Diagnostic Study of 150 Cases of Gliomas Based on Immunohistochemical Profile

Nadeem Reyaz[1], Muhammad Ejaz Butt[3], Muhammad Bahadur Baloch[4], Moniba Zafar[1], Ali Afzal[1] and Naveed Lodhi[2]

ABSTRACT

Objective: To present an overview of the relative frequency of gliomas in the light of expression of biomarkers relevant to the tumor grade.

Study Design: Descriptive study

Place and Duration of Study: This study was conducted at the Department of Pathology, Gulab Devi Teaching Hospital Lahore from February 2018 to December 2020.

Materials and Methods: 150 glioma samples were obtained from the pathology department of Lahore General Hospital by convenience sampling techniqueto be evaluated histologically for grading of the types of a tumor subsequent to hematoxylin & eosin staining. For tumor markers, immunohistochemistry was performed to assess the levels of glial fibrillary acidic protein (GFAP), nestin, and vimentin. The basic and immunostained sections were then assessed by three pathologists deprived of the pathological and clinical data of patients` parameters in a blinded manner. The outcomes of staining were semi-quantitatively scored. The data of incidence rates by histologic type, age, and gender was collected.

Results: The mean age of patients with gliomas was 21.71±16.9. The benign astrocytoma was the most frequent glioma making 52% of all the tumors. The males were on an average 59.34% and the females were 40.66%. The expression level of GFAP staining was considerably lower in high-grade glioma tissues as compared to low-grade glioma tissues. The majority of gliomas revealed moderation expression of nestin around 53.4%. The mean score of vimentin was 8.3 indicating its high expression for the grade IV gliomas.

Conclusion: The expression of biomarkers for gliomas correlated with the grade of the tumor. The mean age revealed gliomas occurring in the younger age group without a major difference in gender distribution.

Key Words: Gliomas, Astrocytoma, Immunohistochemistry, Biomarkers, Glial fibrillary acidic protein, Nestin, Vimentin

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INTRODUCTION

Glioma exist as the predominant type of adult chief glial tumors of the central nervous system. Despite being <1% of the brain tumors, glioma have an association with high mortality within first twenty-four months following the diagnosis.[1,2,3] The poor prognosis associated with glioma is attributed to their high capability of unregulated mitosis and metastasis. Their origin is assumed from the supporting glial constituents of CNS i.e. astrocytes, oligodendrocytes, and ependymal cells.[2] Based on the origin, the glioma is classified based on the World Health Organization 2007 recommendations into numerous morphological subtypes corresponding to the overall appearance of the tissue of origin: oligodendroglioma, astrocytoma ependymoma, and mixed oligoastrocytoma.[4]

A histopathological evaluation has been traditionally utilized for determining the types, subtypes, and tumor grade of gliomas. WHO categorized them into I, II, III, and IV grading criterion based on the level of malignant changes in the perikaryon.[5] The grading of diffuse glioma relies on certain relatively subjective features, for example, the existence or absence of atypical nuclei, endothelial proliferation, high cellular content, mitosis, and necrosis.[1]Despite the criterion, there is subjective variability of astroglial neoplasia which affects the assessment of the survival rates within grades significantly. The histopathologists, therefore, investigated the markers of the related subpopulation of

[1.] Department of Pathology / Biochemistry[2], Al-Aleem Medical College, Lahore.
[3.] Department of Pathology, Amna Inayat Medical College, Lahore.
[4.] Department of Anatomy, Shalamar & Dental College, Lahore.

Correspondence: Dr. Nadeem Reyaz, Associate Professor of Pathology, Al-Aleem Medical College, Lahore.
Contact No: 0300-4232570
Email: drnadeemreyaz@yahoo.com

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cells. Recognition of such analogous markers would greatly benefit diagnostics.[6]

Biomarkers like GFAP and vimentin are intermediate filament (IF) of class III category whereas nestin is a class IV IF with significance in assessing the prognosis of patients with gliomas.[7,8]GFAP was isolated and specified for the first time by Eng and Bignami in 1969 as an intermediate filament protein III constituent of glial cells` cytoskeleton. It is being applied as a biomarker ever since.[6]Nestin was initially designated as a progenitor neural stem cell marker and it has been revealed to completely down-regulate to the extent of extinction during differentiation into mature cells.[7] Vimentin has attained more consideration as the biomarker for the epithelial transition into mesenchymal cells responsible for the attainment of the characteristic tumor based invasiveness and mobility.[8,9,10]

The expression of GFAP, vimentin, and nestin in gliomas differs substantially in terms of their metastatic activity.[6] The rationale of our study was to associate the expression of these biomarkers through immuno-staining in gliomas with histopathological diagnosis and correlate our findings with the age and gender distribution along with the relative frequency of the tumors. Knowledge of variation in the expression of these biomarkers may highlight their regional significance for clinical utilization as markers for diagnosis.

MATERIALS AND METHODS

Hundred and fifty cases of space-occupying lesions previously diagnosed as primary neurological tumors at Pathology Department, General Hospital, Lahore were selected by convenience sampling technique from February 2018 to December 2020. Endorsement of the study was obtained from the institutional ethical committee. Tumor masses were evaluated histologically for glioma diagnosis and grading of their types following hematoxylin & eosin staining. All tumors other than gliomas were excluded from the study.

Deparaffinized slides stained with hematoxylin-eosin for tumor grade assessment and were utilized for immunohistochemistry.

A mouse monoclonal antibody for cytoskeletal filaments and interstitial proteins against GFAP. The cellularity and cytomorphological features were evaluated located in the neoplastic representative area under magnification of X40.[1,3] For nestin immunohistochemistry (IHC), an avidin-biotin-peroxidase complex method was utilized with 1:150 dilution from the monoclonal antibody of mouse against human nestin and mouse VIM Elisa kit for Vimentin IHC.[7]

The patient data was gathered from hospital record for analysis of frequency of age, gender and tumor distribution. The basic and immunostained sections were assessed by three pathologists deprived of the clinical and pathological data of patients` parameters in a blinded manner. The results of staining were semi-quantitatively scored based on Bei, Huang scoring.[1]

The immunoreactive score (IRS) for GFAP, nestin, and vimentin was determined by the estimation of the percentage of the immunoreactive cells along with an estimate of the staining intensity. The percentage of cells was scored as follows: 1 (0-49 % tumor cells stained), 2 (50–74 % tumor cells stained), and 3 (75–100 % tumor cells stained). The intensity of staining was coded as follows: 0= negative staining, 1 = staining weak, 2 = staining moderate, and 3 staining strong. Then the two subsequent scores were multiplied and the mean was classified into two groups: low expression (0–4.5) and high expression (4.5–9).[1]

Statistical Analysis: Data was evaluated through SPSS version 21. Chi-square test was applied to correlate the GFAP score with the grades of the tumors with the biomarkers and to trace the relation between grading of the tumor to age and gender distribution. The p-value of > 0.05 was considered statistically significant.

RESULTS

In a total of 150 cases of space-occupying lesions intra-cranially, 89 cases included males and 61 were females, with a male / female ratio of 1.46:1 as depicted in Table-1. Of all the 150 cases, benignglial tumors had relatively high frequency than tumors with malignant characteristics with p-value = 0.12 (Figure -1). The benign astrocytoma was the most frequent glioma making 52% of all the tumors; 23 cases were found underneath the age of twenty, while most of the cases (52) were found in the third decade. The mean age of patients with glioma was 21.71±16.9. A moderate number of tumors were found in the fourth and fifth decades, with numbers 12 and 20 respectively. Aprecipitous up surge in the number of cases in the sixth decade was noticed. These observations are depicted in Table2. Astrocytoma was found to be the most frequent tumor, ependymomas ranked second, whereas oligodendro-gliomas and glioblastomas multiforme had third and fourth respective prevalence (Table-3, Figure 3)

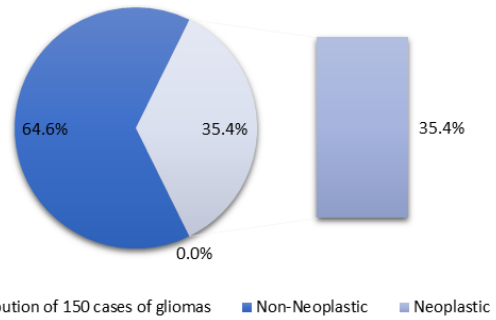

Figure No.1: Distribution of 150 cases of gliomas

Table No.1: Age and Sex distribution of 150 cases of gliomas.

Age group (years)	Sex		Total
	Male	Female	
0 – 9	3	2	5
10–19	11	7	18
20–29	32	17	52
30 – 39	4	9	12
40 – 49	11	7	20
50 – 59	22	17	37
60 – 69	6	2	8
Total	89	61	150

Table No.2: Relative frequency of the different types of neuro-epithelial tumors.

Types of Tumor	Sex*		Total	(%)
	Male	Female		
1.Benign Astrocytomas	52	26	78	52%
2.Anaplastic Astrocytomas	21	11	32	21%
3. Ependymomas	12	10	22	15%
4. Oligodendrogliomas	2	8	10	7%
4. Glioblastomas Multiforme	2	6	8	5%
Total	89	61	150	(100%)

*Difference between males and females for all age groups (P = 0.39)

Of the total 150 cases, 70 were of astrocytic tumors, 12 ependymoma cases, and 6 glioblastoma multiforme were positive for GFAP. Four cases of oligodendroglioma were negative for GFAP along with 1 case of GFAP-positive oligodendrocytes. The GFAP stain revealed intense immunostaining to the subependymal and subpial networks of glial fibers. The expression level of GFAP staining was considerably lower in high-grade glioma tissues as compared to low-grade glioma tissues and normal brain tissues (Figure - 2). In the cases of the grades I & II glioma, the GFAP had a high expression average score >7 whereas tumors of III & IV grading had expression scores between 3-4. Double immunostaining with anti-vimentin and anti-GFAP antibodies emphasized the occurrence of both antigens in the same zones of the tumor (Figure 4)

The nestin expression was not found in 2.1% of the samples, 18.3% revealed low expression, and the majority revealed moderation expression 53.4% whereas 26.2% had high levels of expression. The average IHC nestin score for astrocytes was 5.3 with medulloblastoma 7.2, and 8.7 for glioblastoma multiforme. The nestin was found negative for ependymoma, oligodendroglioma, and choroid plexus papilloma. The vimentin was negative for oligodendrocytomas, however, its positivity increased with the grading of malignancy (p =0.414) (Table 3).

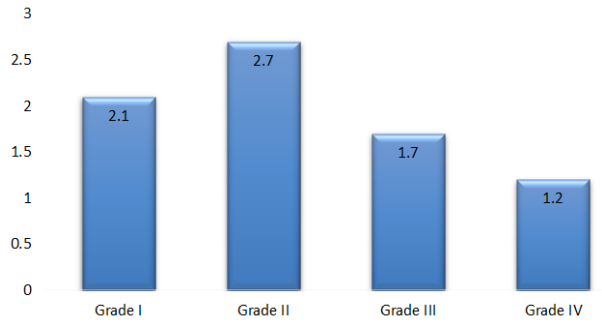

Figure No.2: Glial Fibrillary Acidic Protein Score

Figure 1 Mean Glial Fibrillary Acidic Protein Immunoreactive Score of gliomas from I to IV grading based on Bei, Huang (1) scoring system. The mean score is the sum of the percentage of immunohistochemistry staining and intensity staining of individual grade.

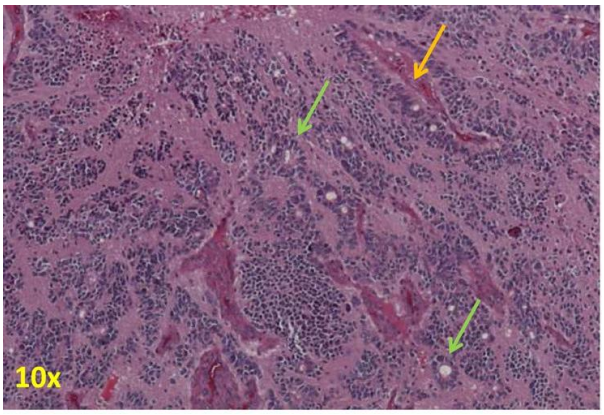

Figure No.3: Hematoxylin and eosin (H&E) staining showing anaplastic ependymoma features. Formation of true rosettes (green arrows) surrounding the microvascular proliferation within ependymal tumors, usually signifies anaplastic transformation which is characteristic of ependymomas. Pseudo palisading necrosis, characterized by a garland-like structure of hypercellular tumor nuclei lining up around irregular foci of tumor necrosis (yellow arrow)

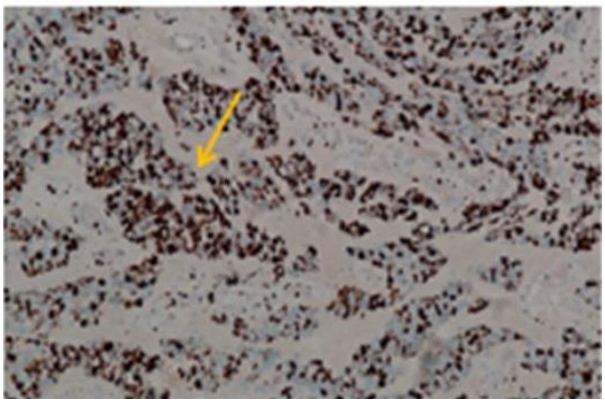

Figure No.4: Photomicrographs of Ki-67, vimentin, GFAP, and EMA immunostaining of the ependymal tumor (yellow arrow). Ki-67 immunostaining indicates a high proliferation index in the tumor (70%)

Table No.3: Correlation of Immunohistochemistry score with the expression of vimentin

Grades	Vimentin IHC Score (mean)	Vimentin Expression
Grade I	3.4	Low
Grade II	3.5	low
Grade III	5.5	Moderate to high
Grade IV	8.3	high

DISCUSSION

Gliomas are the principal tumors of the central nervous system with high morbidity and mortality.[1,6] Glioblastoma account for the majority of gliomas responsible for mortality.[11] However, the incidence of benign vs malignant gliomas varies considerably in the literature.[12]

There were more benign gliomas diagnosed in the 150 cases on histopathology. The mean age of most frequent gliomas diagnosed in our study was similar to the reported age with a peak of benign astrocytomas commencing in the twenties. However, the number of malignant tumors was less, particularly of glioblastoma multiforme, peaking at 50s contrary to 60s and 70s documented by Davis (2018).[13] The high frequency of benign tumors in children and adolescents is commensurate with the possible childhood exposure to the 30 kHz to 300 kHz frequency range of the radiofrequency electromagnetic fields. The intermittent occurrence, on the other hand, debunks the notion of genetically associated risk factors despite the advancement in molecular genetics. The immense inconsistency in the occurrence of types of gliomas is, therefore, bewildered by variations in approach to histopathological diagnosis, medical imaging, and surveillance. Forthcoming studies associating genetic, environmental, and regional lifestyle risk factors amongst and within countries will be significant to comprehend these differences.[13]

Ionizing radiation through mutations and DNA damage has the potential to induce oncogenesis. Early exposure to ionizing radiation as early as the first 7-9 years, either therapeutic or environmental, contributes to neurological tumor formation through aberrations in the cell cycle. Various new risk factors discovered in the past decade besides the neutral stem-like cells in the subventricular zone give rise to atleast a subset of gliomas. Moreover, transformed blood immune cells profile with CD4+ T-cell deficiency and mutation of the isocitrate dehydrogenase gene have been associated with gliomas.[2, 14]

Most epidemiological studies reveal that, along with the differences in geography, glioma incidence varies by age, sex, origin, and tumor subtype.[7] According to Molinaro, Taylor (2019)[2], women bear 50-60% less incidence of developing gliomas at all ages. The more incidence of cancer in men and its probable association with hormone lacks proper scientific evidence. Proposing unidentified risk factors being the root-cause.[2] The more occurrence at the young age could be the new norm of exposure to the cellular non-ionizing radiofrequency electromagnetic fields.[12, 13]

The interobserver and intraobserver variability in the histopathological diagnosis of glioma belies the exceptional limit of its utility. This led to the advancement in the new diagnosis and extensive research on the utilization of biomarkers for therapeutic and prognostic strategies.[7]There is substantial association of the expression of biomarkers with clinical outcome.[6] GFAP is recurrently designated for the visualization of astrocytes and tumors of glial origin. Glial fibrillary acidic protein (GFAP) is recurrently used as a dependable marker for visualization of astrocytes and glial-derived tumors.[4] The isolation followed by the development of antibodies has paved way for GFAP positivity assessment using immunohistochemical staining.[6] GFAP is the indicator of glial cells differentiation. The more pronounced the staining of GFAP is believed to mark less malignant and more differentiated tumors. The mature astrocytes and radial glia, therefore, express more GFAP.[1]

The evident levels of GFAP in the serum of grade II astrocytoma patients were significantly higher which could be attributed to the higher rate of cellular mitosis of astrocytes.[6, 15]Furthermore, there is formation of aggregates in astrocytes with the excessive translation of GFAP genes leading to neurodegeneration.[10]The lower expression of GFAP in dedifferentiated tumor of grade IV could be the consequence of decrease stimulatory response of glial cells to endocrine hormones of progesterone and dihydroprogesterone on GFAP gene.[16]A few examinations exhibited reformist loss of GFAP articulation with ascending astrocytoma grade similar to our findings.[6]

The expression of nestin and vimentin demonstrates an undifferentiated, more stemcell-like condition of these cells.[10] The vast majority of the gliomas that communicated elevated levels of nestin were high-grade gliomas.[17] The association between nestin expression and grade of the tumor has been proposed to be linked to dedifferentiation, enhanced motility of cell, ability to invade locally, and augmented malignant potential.[7]It triggers the activity of phosphorylated focal adhesion kinase (pFAK) on the cell membrane. Phosphorylated FAK degrades the transmembrane linker integrin and promotes tumor formation at the cellular level.[18]The outflow of vimentin was greater in high-grade glioma in correlation with low-grade samples. It is a major marker of epithelial-mesenchymal transition (CMT) through up regulating the receptor tyrosine kinase Axl expression and loss of E-cadherin.[8,19] This makes it effective indicator of the malignancy due to its role in regularization of cellular integrity.[20] It is, therefore, indispensable to recognize

the underlying mechanisms at the molecular level. This will further define the biologic behavior of glioma cells, and then and there, take advantage of new beneficial strategies to regulate the progression of glioma.[3]

CONCLUSION

It is basic to look for efficient biomarkers for the prognosis of glioma to manage the clinical treatment including GFAP, vimentin, and nestin.

Author's Contribution:

Concept & Design of Study:	Nadeem Reyaz
Drafting:	Muhammad Bahadur Baloch, Moniba Zafar, Naveed Lodhi
Data Analysis:	Ali Afzal
Revisiting Critically:	Muhammad Ejaz Butt
Final Approval of version:	Nadeem Reyaz

Conflict of Interest: The study has no conflict of interest to declare by any author.

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