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Clinico-Pathological Significance of Cancer Cell Stem Marker Nestin Immunohistochemical Expression in Pediatric and Adult Brain Glioma

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Abstract
Background: Gliomas, the most common CNS tumors, exhibit significant clinical and molecular differences between pediatric and adult populations. Nestin, a neural progenitor marker associated with tumor invasiveness and stem-like properties, has been studied in adult gliomas but less so in pediatric cases. Investigating Nestin's immunohistochemical expression in both groups may reveal age-specific patterns and provide insights into glioma biology and potential therapeutic targets.
Objective: In the present study, we aimed to determine clinico-pathological significance of nestin immunohistochemical expression in pediatric and adult gliomas
Methods: Expression of Nestin in cancer cells was immunohistochemically studied in 128 patients and its association with clinico-pathological features in pediatric and adult gliomas was evaluated.
Results: Among 128 glioma cases (18 pediatric, 110 adult), Nestin expression was significantly higher in adults. Over 80% Nestin-positive cells were observed in 43% of adult tumors versus 12% in pediatric cases. Adult gliomas also showed greater staining intensity and IRS, with moderate-to-strong expression predominant. ($P = 0.05$).
Conclusion: Nestin expression was markedly elevated in adult gliomas, indicating its association with tumor aggressiveness and potential utility as a cancer stem cell marker in age-specific glioma profiling.

Key words: Nestin, Glioma, Neoplastic Stem Cells, Immunohistochemistry, Brain Neoplasms

Introduction

The most frequent primary tumors of the central nervous system (CNS) are gliomas[1], which vary greatly in their histological and molecular features in both adult and paediatric populations[2].
Due to differences in their embryonic origins and tumor microenvironment[3], these tumors represent a broad spectrum of malignancies that exhibit distinct biological behaviors and clinical presentations[4]. Nestin, a class VI intermediate filament protein, has drawn interest among the biomarkers linked to gliomas because of its function in neurogenesis and expression in undifferentiated brain progenitor cells[5].
The expression of nestin, which is mostly found in high-grade gliomas[6], has been linked to the hallmarks of glioma biology[7], including tumor invasiveness, proliferation, and stem-like features. Several CNS cancers, including gliomas, have been shown to express it immunohistochemically[8] higher levels are frequently associated with aggressive tumor characteristics[9]. However, little is known about how it manifests in pediatric gliomas in contrast to adult gliomas[10]. Analyzing Nestin expression may shed light on the pathobiology of gliomas and their developmental variations[11], given the different molecular landscapes of gliomas in these age groups.
The objective of this study is to examine the localization, intensity, and distribution of Nestin's immunohistochemistry expression in tumor tissues in both pediatric and adult gliomas[12]. We aim to improve our knowledge of Nestin's function in the genesis and evolution of gliomas by describing the expression patterns of the protein in different age group[13]. These results could contribute to the larger area of neuro-oncology by laying the groundwork for future studies into age-specific glioma biomarkers and treatment targets[14].

Objective

In the present study, we aimed to determine clinico-pathological significance of nestin expression in pediatric and adult gliomas.

Materials and methods

Ethics statements

The Institutional Ethical Committee IPDM KMU (KMU/IPMD/IEC/2022/8) approved the current study, which complied with the Declaration of Helsinki procedure.

Patients and tissue specimens

This prospective cohort study comprised 128 glioma patients who were admitted to Prime Teaching Hospital between January 2023 and September 2024. Patients who underwent radiation therapy or chemotherapy prior to surgery were not included. The World Health Organization's (WHO) classification of brain tumors served as the basis for the histological diagnosis. The structured proforma contained all of the individual data.

Inclusion Criteria:

1. De novo patients of all age groups with gliomas diagnosed on biopsy in the Pakistani population of Khyber Pakhtunkhwa.
2. All biopsy specimens classified as grade I-IV gliomas based on H&E staining

Exclusion Criteria:

1. Brain tumor patients diagnosed other than glioma on biopsy examination
2. All biopsy specimens having any kind of artifacts
3. Patients who received preoperative chemotherapy and/or radiotherapy were excluded

Optimization of Antibodies

Before all of the glioma samples were finally immunohistochemically stained with Nestin antibody, the optimal staining signal—that is, a clear stain with the highest intensity and the least amount of background staining—was obtained by adjusting the dilution and antigen retrieval times. The sections were left at room temperature for one hour to react with a 1000-times-diluted anti-Nestin antibody (clone 10C2, Vitro China). To recover the antigen, the sections were autoclaved for 10 minutes at 121°C in 0.01 mol/L citrate buffer (pH 6.0) with 0.1% Tween 20.

Evaluation of immunohistochemical staining

Microscopic analysis of the IHC slides revealed that the tumor cells expressed nestin cytoplasmic immunostaining, which was deemed to be positive..

The Immuno Reactive Score (IRS) is a semi-quantitative evaluation that is calculated by multiplying the staining intensity by the percentage of tumor cells that have been positively indicated. The percentage of positive cells is rated as: [0] no positive cells; [1] <10% positive cells; [2] 10-50% positive cells; [3] 51-80% positive cells; and [4] >80% positive cells. Staining intensity is scored as [0] negative, [1] mild, [2] moderate [3] intense. The percentage of positive cells score is multiplied by the staining intensity score to calculate an IRS value between 0-12 which is interpreted as 0-1= negative, 2-3week staining, 4-8= moderate staining, and 9-12 = strong staining.

Statistical analysis

SPSS version 23.0 software was used for all statistical analyses (SPSS Inc., Chicago, Illinois). Either Fisher's exact test or Pearson's $\chi 2$ test, if applicable, were used to evaluate the associations between nestin expression and clinicopathological characteristics. Differential significance was defined as a P-value of less than 0.05. P-values that are presented are all two-sided.

Results

Patient characteristics

There was a total of 128 cases included in this study. The age range was from 3-75 years with a mean age of 33.6 years (S.D ± 17.06 years). Demographic details of all patient are given in Table 1 to 4.

Table 1: Frequency of glioma patient according to age groups.

	Frequency	Percent
Pediatric	18	14.06
Adult	110	85.04
Total	128	100

Table 2: Frequency of glioma patient according to gender:

Gender	Pediatric	Adult	Total	P value
Male	14	71	85	
Female	4	39	43	0.271
	18	110	128	

Table 3: Frequency of glioma patient according to site:

Site	Pediatric	Adult	Total	P value
Supratentorial	12	87	99	0.243
Infratentorial	6	23	29	
	18	110	128	

Table 4: Frequency of glioma patient according to literality:

Literality	Pediatric	Adult	Total	P value
Left	8	49	57	0.491
midline	3	9	12	
Right	7	52	59	
	18	110	128	

The analysis of Nestin-positive cells revealed notable differences between pediatric and adult gliomas. Adult glioma cases predominantly showed a higher percentage of Nestin-positive cells, with 43% of cases demonstrating >80% positivity. In contrast, only 12% of pediatric cases reached this expression level. The lower percentage ranges (<10% and 10–50%) were almost exclusively observed in adults, indicating more variable expression in this group shown in figure 1. These findings suggest that adult gliomas tend to exhibit a higher degree of Nestin-positive cell proliferation compared to pediatric gliomas.

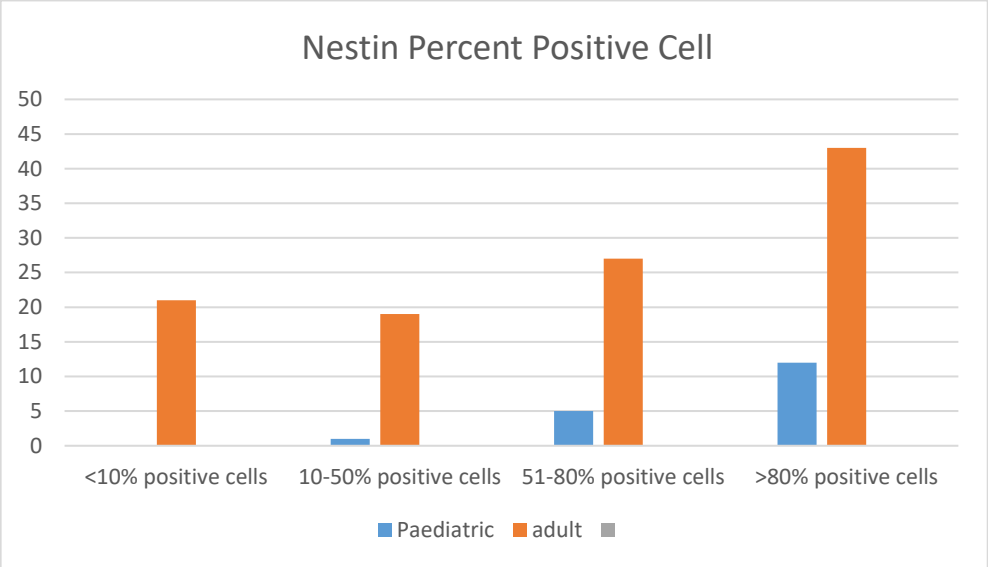

Figure 1: Expression of nestin percent positive cells in pediatric and adult glioma patients

Staining intensity further emphasized this disparity. A majority of adult gliomas demonstrated moderate (57%) to strong (33%) Nestin expression. In pediatric gliomas, moderate intensity was the most common (11%), while strong intensity was infrequent shown in figure 2. This pattern supports a more robust activation of Nestin-related pathways in adult tumors, which may reflect their more aggressive or differentiated phenotypes.

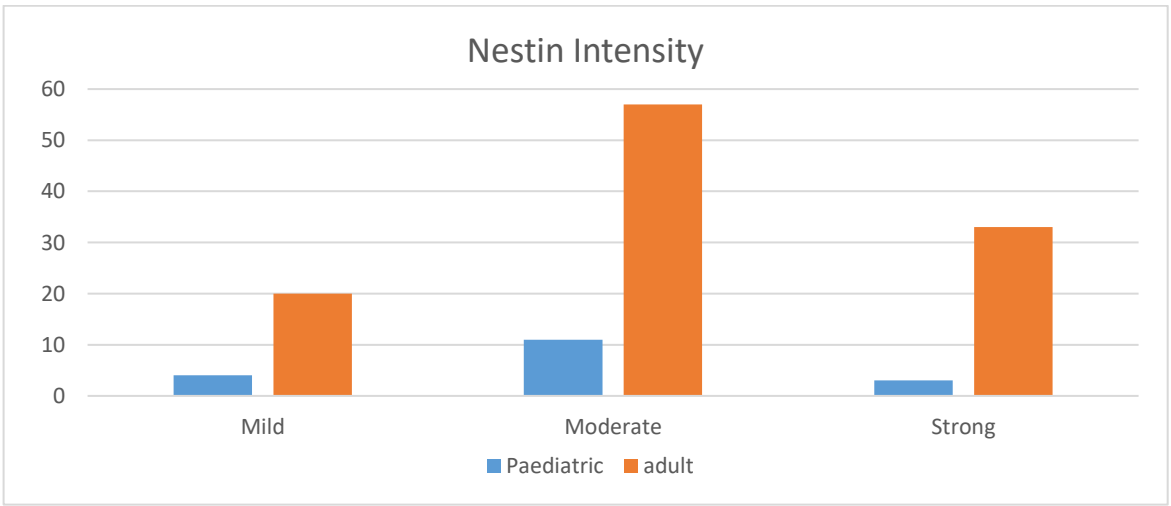

Figure 2: Expression of nestin Intensity in pediatric and adult glioma patients

The IRS distribution again showed a trend of elevated Nestin expression in adults. Moderate staining was the dominant category in adults (58%), followed by mild (28%) and strong (21%) staining. In pediatric gliomas, the majority fell within the moderate staining range (16%), with minimal cases showing strong staining shown in figure 3. These results align with intensity data, reinforcing the observation that adult gliomas exhibit higher overall Nestin expression.

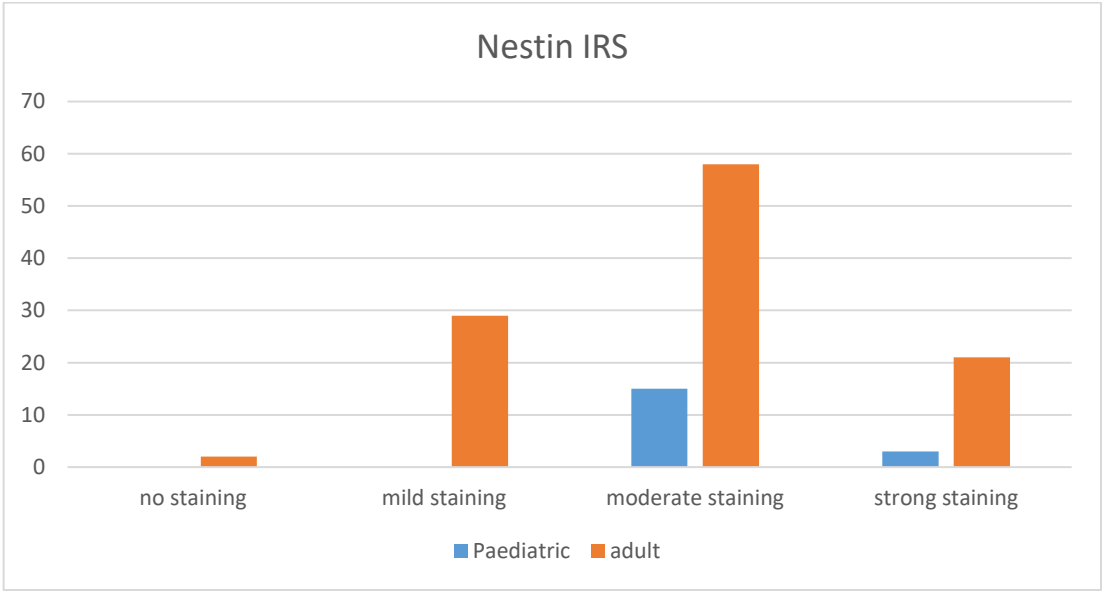

Figure 3: Expression of nestin IRS in pediatric and adult glioma patients

Discussion

Neural stem cells and undifferentiated states in a variety of malignancies, including gliomas have long been linked to the class VI intermediate filament protein nestin[15], examining its immunohistochemistry expression in both pediatric and adult gliomas with an eye toward possible associations with pathological and clinical factors was the goal of this investigation[16]. According to our research, there was no statistically significant correlation between Nestin expression in either group and either gender, tumor laterality, or tumor site. This is in line with earlier research, like that conducted by Ben G. McGahan et al[17]and Strojnik et al[18]. Which found that nestin expression is more closely associated with the biological features of gliomas than with tumor location or patient demographics. These findings highlight Nestin's function as a biomarker that is unaffected by outside clinical variables and highlights its connection to intrinsic tumor behavior[19].

The statistically significant correlation between the immunoreactivity score (IRS) and the proportion of Nestin-positive cells in bothpediatricand adult gliomas was a noteworthy finding of our study. Given that tumors with a higher proportion of Nestin-positive cells were linked to higher IRS values, this study emphasizes the biological significance of Nestin expression levels. Similar patterns have been shown in earlier research, such as that conducted by Strojnik et al[18]., wherein Nestin expression was linked to higher tumor grades, enhanced proliferative potential, and a phenotype resembling stem cells[20]. This implies that underlying tumor aggressiveness and plasticity are reflected in the positive connection between IRS and Nestin-positive cells[21].

Our results are in line with research indicating that, despite the fact that pediatric gliomas have different genetic and molecular profiles than adult gliomas, Nestin's function as a glioma stemness marker is constant[22]. According to research by Sottoriva et al., gliomas in children and adults share stem-like tumor niches that are marked by strong Nestin expression[23]. This lends credence to the idea that Nestin could function as a universal indicator of glioma biology at any age. This correlation's constancy across age groups highlights Nestin's potential use as a biomarker for glioma characterization in a range of clinical settings.

Furthermore, the strong correlation between IRS and Nestin-positive cells might be due to Nestin's role in angiogenesis, hypoxia adaption, and invasive potential—processes that are essential to the development of gliomas[24]. Nestin expression is frequently elevated in hypoxic tumor areas, where it promotes invasion and survival, according to a prior study by Mertens et al.[25] given that these regions most likely reflect areas of enhanced malignancy and adaptability to unfavorable microenvironmental settings, this could help to explain the raised IRS in tumors with a large percentage of Nestin-positive cells. These results provide a mechanistic relationship between high Nestin expression and tumor aggressiveness and are consistent with research investigating the role of Nestin in tumor vascularization and its association with invasive characteristics[26].

Although there have been prior reports of gender variations in glioma incidence[27], it is interesting to note that the lack of relationships with clinicopathological characteristics like laterality and gender highlights the intrinsic, tumor-specific nature of Nestin expression. Although demographic and anatomical factors affect tumor incidence and appearance(28), they do not seem to have an impact on Nestin expression patterns, according to glioma studies like those from the Glioma Atlas Project[29]. The notion that Nestin's function is based on the cellular and molecular dynamics of the tumor rather than the patient's outward features is supported by this observation[17].

Conclusion:
Our results, taken together, contribute to the increasing amount of data showing that Nestin is a significant indicator of glioma aggressiveness, independent of patient characteristics. Nestin-positive cells' significant correlation with IRS validates its use as a marker of tumor growth and adaptability.

Recommendations
The molecular processes controlling Nestin expression and its functional significance in glioma subtypes should be further explored in future studies. Furthermore, research on therapeutic techniques that target Nestin-positive tumor cell types may offer fresh methods for treating gliomas in both adult and pediatric age groups.

Limitations
Small biopsy specimens and difficulty performing brain parenchymal lesion biopsies were factors in the diagnostic difficulties.

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