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Article

Nestin and WT-1: Potential Interplay in Astrocytoma Tumor Biology

Asma Jalbani[1], Parisa Bashir[2], Prih Bashir[3,*], Javeria Tunio[4], Farah Siraj[1], Arpna Nihal[5]

[1]Pathology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi, Pakistan
[2]Liaquat University of Medical and health sciences, Jamshoro, Pakistan
[3]Government of Sindh, Karachi, Pakistan
[4]Chandka medical hospital, Larkana, Pakistan
[5]Sindh Blood Transfusion Authority, Karachi, Pakistan
[*]Corresponding author: Prih Bashir, drprihbashir@gmail.com

Abstract

Background: Glial cells are the origin of gliomas, a diverse group of brain tumors including Astrocytoma, Oligodendrogliomas and Ependymoma, classified based on their cellular origin. The St. Anne/Mayo grading system evaluates key histological features such as necrosis, endothelial proliferation, mitotic activity and cellular atypia to determine tumor grade and guide treatment. Although less common than other cancers, CNS malignancies cause significant morbidity and mortality, with thousands of new cases and deaths reported annually worldwide. Objective: The purpose of the current study was to examine the relationship between immunoexpression of the Nestin protein and WT-1 gene and various grades of Astrocytoma. Methods: A cross-sectional study was carried out at the Pathology Department of Basic Medical Science Institutes, Jinnah Postgraduate Medical College in Karachi, Sindh, Pakistan, from January 2019 to December 2022. There were 60 Astrocytoma cases in this investigation. All the cases of various grades of Astrocytoma were received in the department of Pathology, Basic Medical Sciences Institute, Jinnah Post Graduate Medical Center, Karachi were deeply reviewed and were included in this study. Results: Sixty examples of Astrocytoma were immunostained with WT-1 and Nestin. 86.7% of Astrocytoma cases had Nestin positivity, while 93.3% had positive WT-1 immunostaining. It was shown that there was a significant association ($p<0.001$) between the score of the both immunomarker Nestin and WT-1 and the grade of the tumor, with a higher score for high-grade (grade III and grade IV) and a lower score for low-grade (grade I and grade II) Astrocytoma. Conclusion: Nestin and WT1 immunoexpression is positively correlated with higher tumor grade and enhanced mitotic activity, suggesting that they may be useful prognostic indicators in Astrocytoma. Additionally, their strong association with tumor aggressiveness highlights Nestin and WT1's potential as therapeutic targets as well as prognostic factors, opening the door to more individualize and efficient Astrocytoma management treatment plans.

Keywords

Astrocytoma, Nestin, WT-1, Tumor grade, Prognosis, Immunoexpression

Article History

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1. Introduction

Astrocytoma are a type of cancerous tumor that develops from astrocytes, which are glial cells with a star-shaped morphology that support the brain's structural and functional integrity. Apart from astrocytes, the brain is also home to Oligodendrocytes, which create the myelin coating that surrounds nerve fibers and Ependymal cells, which line the ventricles of the brain and aid in the creation and movement of cerebrospinal fluid. The proper operation of the brain and spinal cord depends on these cells, which are collectively referred to as glial cells. These glial cells give rise to a class of cancers called gliomas. Astrocytoma, Oligodendroglioma, and Ependymoma are the three primary forms of gliomas; each is called for the particular kind of glial cell that gives rise to it [1].

An approach that is frequently used to evaluate the neuropathological features of gliomas is the St. Anne/Mayo grading system. It assesses four important histological characteristics: necrosis (areas of dead tissue), endothelial proliferation (abnormal growth of blood vessel cells), mitotic activity (rate of cell division) and cellular atypia (abnormal cell appearance). The tumor grade, which indicates the aggressiveness of the glioma and aids in guiding prognostic and therapeutic decisions, is determined by the presence and combination of these characteristics [2].

Despite being less frequent than lung or breast cancer, malignancies of the central nervous system (CNS) significantly affect rates of morbidity and death. Each year, they cause a significant number of new cases and fatalities. With 251,329 fatalities and 308,102 new cases per year, CNS malignancies are the 20th most common malignancy worldwide [3].

1.1 WHO Histological Classification

1.1.1 Diffuse Astrocytoma

Diffuse Astrocytoma grade II: only nuclear atypia. Anaplastic Astrocytoma grade III: Nuclear atypia and focal/dispersed anaplasia. There is noticeable mitotic activity and growth. Glioblastoma Multiformis grade IV: Necrosis, mitoses, nuclear atypia, or microvascular proliferation (WHO).

1.1.2 Pilocytic Astrocytoma

WHO grade I is corresponding to Pilocytic Astrocytoma. Pilocytic Astrocytoma's Microscopic Characteristics suggests no final grade allocation for Pilomyxoid Astrocytoma at this time. Grade I Subependymal giant cell Astrocytoma. Grade II Pleomorphic xanthoastrocytoma. Grade III Anaplastic pleomorphic Astrocytoma.

1.2 Nestin

The Nestin gene on chromosome 1 encodes the Nestin protein, which is expressed by a variety of stem cell and tissue types. Although it is frequently linked to neural stem cells, this intermediate filament protein is found in a variety of other cell types, which reflects its function in cell development and plasticity. These consist of umbilical cord blood cells, odontoblasts, heart muscle cells, kidney progenitor cells, liver cells, testicular cells, hair follicle sheath cells and skeletal muscle cells. Nestin's broad expression indicates that it has a role in the initial phases of tissue regeneration and cell differentiation in a variety of organ systems [4]. These intermediate filaments are critical for supporting organelles, tissue growth and regeneration and cytoskeletal elements and preserving the mechanical integrity of cells [5].

As an intermediate filament protein, Nestin is essential for controlling the mechanical characteristics of cells, especially their structural integrity and stiffness. Nestin expression has been linked to decreased cellular stiffness in cancer cells, which improves the cells' capacity to migrate and deform through surrounding tissues. This decrease in stiffness promotes tumor growth and metastasis by making it easier for cancer cells to invade and spread to far-off locations [6].

Nestin is essential for increasing the cytoskeleton's pliability and flexibility, which enables the cell to move through tissues and adjust to mechanical pressures. According to studies, cells lacking Nestin become more rigid and less motile, which severely hinders their capacity to proliferate and develop metastases. Because of this decrease in cellular stiffness, Nestin may be a target for treatment approaches meant to stop the migration of cancer cells. A promising strategy to stop the spread of cancer may be to target and alter Nestin expression in cancer cells, which may lessen the cells' capacity to invade neighboring tissues and disseminate [7].

1.3 Wilms' Tumor

In 1899, Max Wilms discovered that the gene causing Nephroblastoma, a form of kidney cancer that mostly affects children, is caused by WT1 (Wilms' Tumor 1). Research on WT1 has greatly increased since this first discovery, concentrating on its genetic makeup, expression patterns and cellular localization in addition to its function in tumor growth. WT1's significance extends beyond cancer biology, as research has shown that it is involved in a number of physiological processes, such as organ development, cell differentiation and apoptosis [8].

The Wilms tumor gene has been discovered on chromosome 11p13 [9,10]. A transcription factor that is essential for controlling the expression of genes involved in cell division and proliferation is encoded by WT1. Controlling the growth and development of different cell types, especially during embryogenesis, requires this protein. Although its

dysregulation has been connected to cancer and other clinical diseases, the WT1 transcription factor aids in maintaining appropriate cellular function and tissue formation by affecting the transcription of target genes [11,12]. In addition to soft tissue sarcomas including rhabdomyosarcoma and malignant peripheral nerve sheath tumors, WT1 has been linked to a number of cancers, including ovarian, breast, leukemia, and brain cancers. WT1 dysregulation in these cancers indicates that it plays a crucial part in the development and spread of tumors, possibly affecting functions like cell division, survival, and metastasis. Its significance as a biomarker and a possible therapeutic target in the treatment of cancer is highlighted by its participation in a variety of tumor forms [13,14].

1.4 Objectives of the Study

To assess and quantify the expression levels of Nestin and WT1 across various grades of Astrocytoma: This objective aims to measure the relative abundance of Nestin and WT1 proteins in Astrocytoma, spanning different tumor grades (low-grade to high-grade).

To investigate the potential correlation between the expression levels of Nestin and WT1 and clinical outcomes, such as tumor grade and patient prognosis: This objective seeks to explore the relationship between the expression of Nestin and WT1 and various clinical indicators, such as tumor grade, patient survival rates, and other prognostic factors. Understanding this correlation may reveal the role these proteins play in tumor aggressiveness and provide insights into how they could be used as predictive biomarkers for patient outcomes, influencing treatment strategies and overall management of astrocytoma patients.

2. Materials and Method

This study conducted a retrospective cross-sectional analysis of histopathologically diagnosed astrocytoma cases. The data was collected from the Basic Medical Science Institute, Jinnah Postgraduate Medical College Karachi histopathology department over a period spanning from October 2019 to September 2022. The study was granted approval by the Jinnah Postgraduate Medical College Ethics Review Board (ERB), ensuring that ethical standards were met in the review and analysis of patient data.

A total of 60 Astrocytoma cases were included in the study, selected using non-probability convenience sampling. The primary objective was to evaluate the expression levels of Nestin and WT1 proteins across these cases. This sampling method allowed for a practical selection of available cases within the study period, while providing valuable insights into the role of these biomarkers in Astrocytoma.

2.1 Immunochemistry

Sections placed on Poly-L-Lysine-coated slides were stained with WT-1 and Nestin. The marker's technique was used in accordance with the manufacturer's instructions. Xylene modifications and a decreasing alcohol concentration were used to dewaxe and rehydrate tissue slices. Tris-Hcl buffer (PH 9.0) and a steamer were used for antigen unmasking. The sections were stained using the Nestin mouse monoclonal primary antibody (catalogued as REF 388M-18, from Cell Marque Corporation) and WT-1 Mouse monoclonal antibody, with a unique catalog number, REF IR055, was obtained from Dako Corporation after peroxidase was blocked. After secondary antibody incubation, sections were coated with Di-aminobenzidine chromogen to enhance visibility. As positive controls, sections of ovarian serous adenocarcinoma for WT-1 and malignant melanoma tissue for Nestin were used. Every slide was evaluated by two pathologists.

2.2 Interpretation

The cytoplasmic expression of the immunomarkers Nestin and WT1 was considered positive when these proteins were detected within the cytoplasm of the tumor cells. This indicates that the proteins were actively expressed and localized in the cytoplasmic compartment, which can be indicative of their involvement in cellular processes such as growth, differentiation and metastasis.

2.3 Immunohistochemical evaluation of Nestin and WT-1

To evaluate the staining further, an immunoreactive score (IRS) approach comprising the intensity score (IS) and the percentage score (PS) was applied. The percentage of cells with a positive stain is indicated by the intensity score, which varies from No staining=0 to Weak staining=1, Medium staining=2 and Strong staining=3. The PS, which has values of 0 for negative tumor cells, 1 for less than 30% positive tumor cells, 2 for 30%–60% positive tumor cells, and 3 for more than 60% positive tumor cells, represents the staining intensity. An immunoreactive score is obtained by multiplying the IS by the PS. A score of 1-3 is considered bad, 4-6 is considered intermediate, and 7-9 is considered high in terms of expressiveness level.

A semi-quantitative scoring system was used to evaluate WT-1 expression. The purpose of this scoring system was to quantify the amount of cytoplasmic staining in cancer cells as a result of WT-1 expression. A score was given to the frequency of WT-1 expression by cancer cells, with 0 representing no expression, 1 representing less than 25% expression, 2 representing 25%–75% expression, and 3 representing more than 75% expression. In addition, cancer

cells were scored 0 for negative expression or 1 for mild intensity depending on the strength of WT-1 staining in comparison to normal glial cells. A score of 2 denoted moderate intensity while a score of 3 marked notable intensity. The frequency and intensity scores were combined to create six indices: 0, 1, 2, 3, 4, 5, and 6. Three categories of these indices were identified: marked (indices 5 and 6), moderate (indices 3 and 4), and negative (indices 1 and 2).

Data collection and subsequent statistical analysis were performed using SPSS version 21**,** a widely used software for data management and statistical computing. To assess the relationships between categorical variables and determine statistical significance**,** the chi-square test was applied. A p-value of ≤0.05 was considered the threshold for statistical significance, indicating that any observed differences or associations were unlikely to have occurred by chance alone.

3. Results and Discussions

Figure 1 shows the Nestin immunoreactivity in relation to the Astrocytoma grades. The Pearson Chi square test revealed a statistically significant correlation between Nestin immunoreactivity and Astrocytoma grades ($p<0.001$) and Figure 2 shows the immunoreactivity in all instances of various astrocytoma grades. Immunoreactivity and astrocytoma grade were significantly correlated, according to the Pearson chi-square test ($p<0.001$).

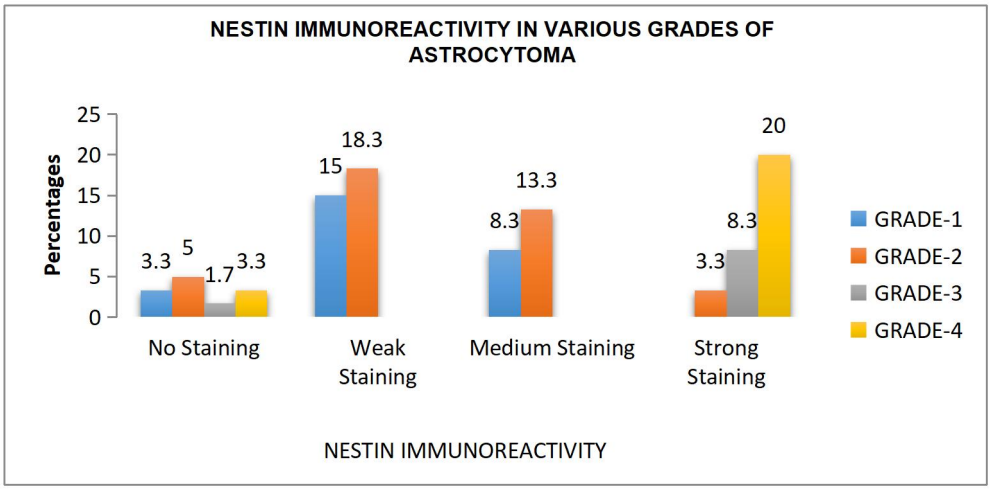

Figure 1. Bar chart displaying Nestin immunoreactivity for various Astrocytoma grades.

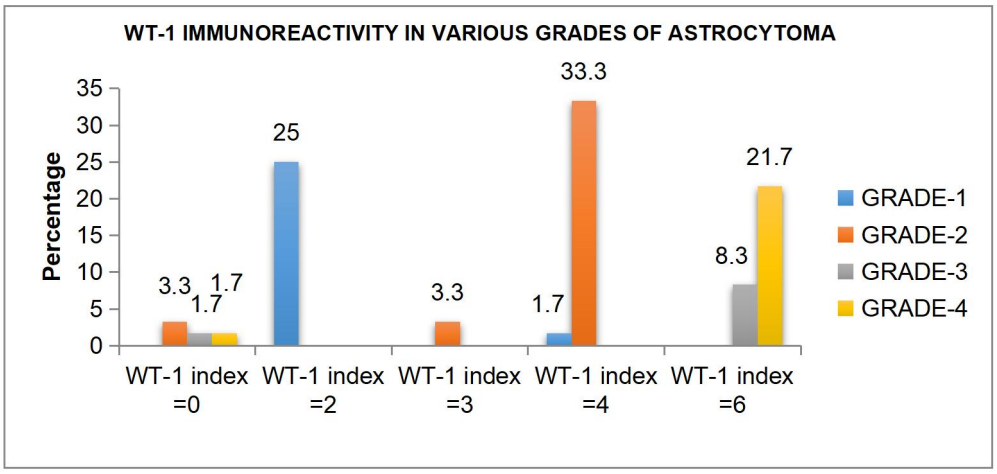

Figure 2. Bar chart displaying WT-1 immunoreactivity for various Astrocytoma grades.

Figure 3 depicts Pilocytic Astrocytoma grade I showing composed of both dense (downward arrow) and loose microcystic (upward arrow) areas and Figure 4 depicts a grade I Pilocytic Astrocytoma showing mild positivity for Nestin immunostaining in tumor cells. Figure 5 depicts Anaplastic Astrocytoma showing increased cellularity significant nuclear pleomorphism and hyperchromasia and increased mitoses and Figure 6 depicts strong Positive Expression of Nestin in Hyper-cellular areas of Anaplastic Astrocytoma. Figure 7 depicts Glioblastoma Multiformis grade IV showing high cellularity with significant nuclear atypia, necrosis areas of hemorrhage and mitoses and Figure 8 depicts Glioblastoma Multiformis grade IV strongly Positive Nestin in tumor cells. Figure 9 depicts Glioblastoma Multiformis grade IV strongly Positive WT-1 and also WT-1 is also positive in endothelial linings of the vessels.

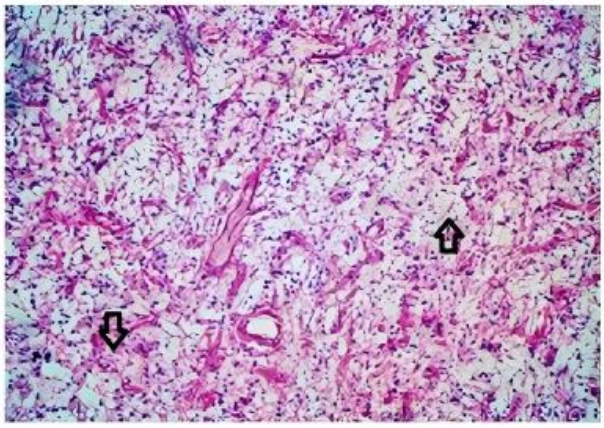

Figure 3. Pilocytic Astrocytoma grade I.

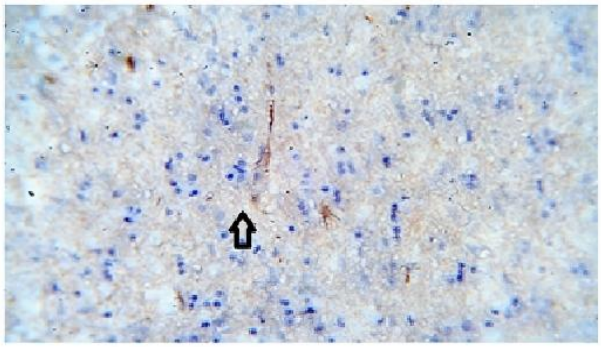

Figure 4. Pilocytic Astrocytoma grade I showing mildly positive Nestin.

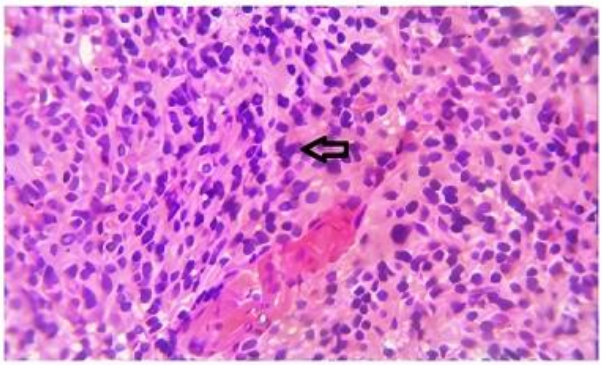

Figure 5. Anaplastic Astrocytoma grade III (H&E X40).

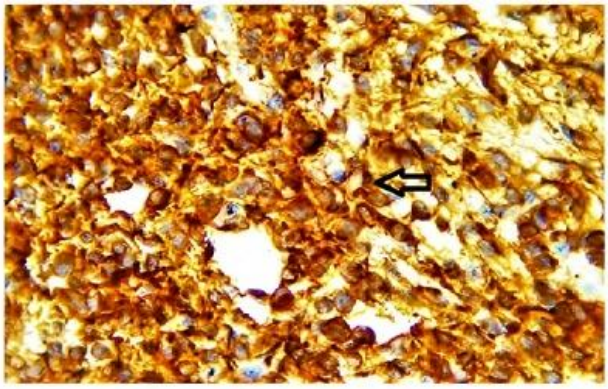

Figure 6. Positive expression of nestin in anaplastic astrocytoma grade III (H&E X40).

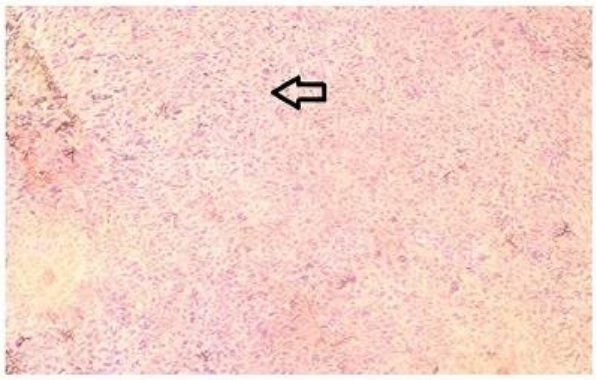

Figure 7. Glioblastoma Multiformis grade IV(H&E X4).

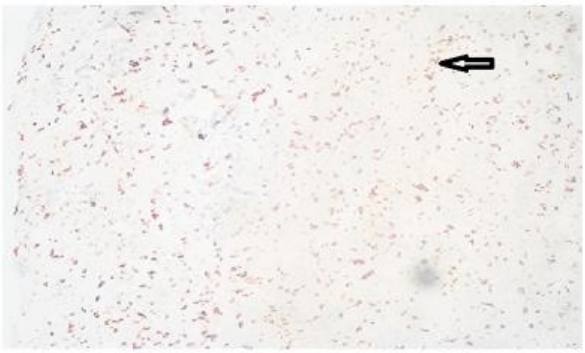

Figure 8. Glioblastoma Multiformis grade IV strongly positive Nestin (X40).

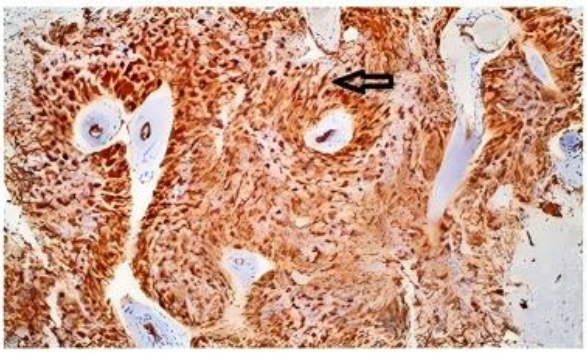

Figure 9. Glioblastoma Multiformis grade IV strongly positive WT-1 (X40).

This study aims to assess the expression levels of Nestin and WT-1 in various grades of Astrocytoma. Additionally, we will explore the potential correlation between these expression levels and clinical outcomes, including tumor grade and patient prognosis**.** We obtained 60 cases of Astrocytoma for our study, of which 40 were low grade and 20 were high grade. Men were more commonly affected by Astrocytoma and the majority of cases occurred in the third and second decades of life respectively.

According to our findings, greater grades of Astrocytoma are linked to higher levels of Nestin immunoreactivity, which suggests that the tumor cells are proliferating more quickly. The study also found a strong correlation between the grade of the Astrocytoma and the immunoreactivity to Nestin. Clinicians may find this information useful in assessing the tumor's aggressiveness and developing effective treatment plans.

Eight (13.3%) of the Astrocytoma in the current study had negative staining. In contrast, a lower percentage of the Astrocytoma in Abdul Kareem et al.'s 2019 study–just 3/48 or 6.25% exhibited negative staining than did ours [15]. According to a finding by Woo et al., more cases 32 (34.8%) than in our study had negative staining. This result may be due to the inclusion of all glioma patients in their study [16].

Of the Astrocytoma examined in this study, 20 (33.3%) had poor staining. A study finds that 9 (18.75%) patients of Astrocytoma had poor staining, which is less than in our sample [15]. According to another investigation, poor staining was found in 22 (23.9%) glioma patients [16]. Furthermore, our study found that 13 (21.7%) of the Astrocytoma had moderate Nestin staining. While Abdel Kareem et al.'s research found that 25 (28.2%) of the Astrocytoma had significant Nestin staining, this number is slightly higher than ours [15].

Of the Astrocytoma in our study, nineteen (31.7%) displayed significant staining. A research shows substantial staining is present in 11 (22.9%) of the Astrocytoma [15]. An et al. report that 35 (28%) of the gliomas have Nestin staining, which is higher than our data because they include all of the gliomas [17].

There was a strong correlation between Nestin immunoreactivity and Astrocytoma grades. Our findings demonstrate a significant Nestin expression in high grade malignancies. Similar results were obtained by Rehfeld et al., suggesting a high association between Nestin immunoreactivity and Astrocytoma grades [18].

According to this study (2/24), 3.3% of grade II Astrocytoma expressed a lot of Nestin. In line with our results, Rushing et al. reported that 1.37% of grade II Astrocytoma have considerable Nestin positivity [19]. Patients with WHO grade II malignancies and high Nestin levels had a significantly worse progression-free survival (PFS) rate [20]. Diffuse Astrocytoma, which has a poor prognosis because of increased Nestin expression, can explain it. Moreover, when Astrocytoma grades rise, higher expression of Nestin is linked to a poorer prognosis for patients, according to Dahlrot et al. [20].

According to our findings, no staining was seen in 2/14 of Glioblastoma Multiformis grade 4 and 1/6 of Anaplastic Astrocytoma grade 3. In the study conducted by Abdul Kareem et al., it was found that out of the five (grade IV) Glioblastoma Multiformis patients, two had moderate staining and three had weak staining. Of the eleven (grade III) Anaplastic Astrocytoma cases, eight had strong staining [15]. In another study, roughly half of all Anaplastic Astrocytoma were found to have poor Nestin positivity [16]. The removal of antigens from the tissue during tissue processing may result in negative staining. There isn't any research that shows that Astrocytoma in grades III and IV exhibits no Nestin staining at all.

Previous research' findings reveal that Glioblastoma (Astrocytoma designated as Glioblastoma Multiformis WHO grade IV) have greater levels of Nestin expression than do low-grade gliomas (WHO grades II–III). Furthermore, a study has demonstrated that when low-grade invasive gliomas (grade II), anaplastic gliomas (grade III), or GBMs (grade IV) are grouped together into a single category, elevated Nestin expression is also associated with shorter survival times [21].

Similarly, WT-1 immunostaining was carried out on each of the 60 Astrocytoma cases. Of these, 56 (93.3%) had positive cytoplasmic WT-1 immunostaining. WT-1 expression index values were greater in high-grade (grade III and grade IV) Astrocytoma than in low-grade (grade I and grade II). The WT-1 score and tumor grade were found to be statistically significantly correlated ($p<0.001$), with higher scores in high-grade (grade III and grade IV) and lower scores in low-grade (grade I and grade II) Astrocytoma. Considerable correlations were found between WT-1 expression and tumor grade. WT-1 index of 6 was present in around 90% (18/20) of high-grade Astrocytoma. There was no low-grade astrocytoma found, with a WT-1 index of 6.

Similar outcomes to our study 100%, 91.24%, 95.9%, and 96%, respectively have been reported in a number of researchs. It was discovered that WT-1 expression was limited to the cytoplasm of cancerous cells [22-24]. WT-1 protein was found in the cytoplasm of WT-1 immunopositive tumor cells, as demonstrated in a study by Oji et al., suggesting that WT-1 may have a role other than transcriptional control, maybe in RNA metabolism as a component [25]. Yokota et al. report that high cellularity and locations where perivascular proliferation was seen in all positive cases were associated with high levels of WT-1 protein expression. These outcomes concur with what we discovered during the current investigation. Based on the findings of this investigation, it is possible that the WT-1 gene plays a significant role in the growth of gliomas and may serve as a marker for the growth of glial tumors [24]. Oji et al. found that Astrocytoma with increased WT-1 protein expression levels also had higher tumor grades [26].

According to a 2009 study by Schittenhelm et al., 52% of diffuse Astrocytoma had positive immunoexpression of WT-1, while more than 75% (score 6) of high-grade gliomas had positive immunoexpression of WT-1 [27]. Bassam et al. discovered a positive link between higher scores and advanced malignancies. grade I patients, with the exception of one, had WT-1 index 2 with mild intensity [28]. Yokota et al. found negative expression in grade II Astrocytoma, which is consistent with our results. Our findings are consistent with those of Mahzouni and Meghdadi, who found primarily moderate WT-1 expression in grade II cases [24,29].

While grade II Astrocytoma reported in a study by Bassam et al. showed a strong presence of moderate expression, which is similar with the findings of the current study [27-28], Schittenhelm et al. saw grade II Astrocytoma and discovered notable expression. The IDH1 mutation in young patients with high-grade Astrocytoma explained the negative expression in grades III and IV reported in this investigation. Additionally, Rauscher et al. noted that older patients had higher levels of positive WT-1 expression than negative WT-1 expression [23]. The current investigation discovered that every case of negative WT-1 expression were also noted in pediatric patients.

Oji et al. reported that WT-1 was present in some glioblastoma cell lines but not in others [25]. This may explain for the absence of WT-1 expression in one of the cases in this investigation. Conversely, Bassam et al. found only weak expression in one glioblastoma case and no negative expression in any of the cases they examined [28]. Both the Rauscher et al. and Schittenhelm et al. studies discovered that Astrocytoma had a considerably greater WT-1 expression level than Oligodendroglioma. The findings of this investigation imply that WT-1 is involved in the Astrocytoma differentiation of brain glial cells [23,27].

WT-1 can therefore be utilized as a diagnostic tool and to differentiate Astrocytoma from Oligodendrocytoma malignancies. Another investigation found that WT-1 was useful in differentiating reactive gliosis from tumor recurrence when treatment-related alterations such necrosis and reactive gliosis were present [22]. These topics were left out of the study because the focus was solely on Astrocytoma tumors. The current investigation discovered a strong relationship between various tumor grades and the mean WT-1 score.

Our study identified a significant positive correlation between WT1 and Nestin expression in Astrocytoma, indicating that these markers may be functionally interconnected and contribute to tumor progression. Their co-expression not only reinforces their potential as prognostic biomarkers but also suggests that they may serve as targets for therapeutic intervention. Given WT1's role as a tumor-associated antigen and Nestin's association with cancer stem-like properties, both markers present promising avenues for targeted therapies.

Importantly, the immunogenic nature of WT1 highlights the potential of incorporating WT1-based immunotherapy into astrocytoma treatment strategies. Future studies should investigate the efficacy of WT1-targeted vaccines, T-cell therapies, or combination immunotherapies in patients stratified by WT1 and Nestin expression levels. Elucidating the molecular interplay between these markers could also inform the development of novel, biomarker-guided immunotherapeutic approaches aimed at improving patient outcomes in Astrocytoma.

Recent studies have explored the use of Nestin cells in treating various diseases. For example, Nestin cells from Peyer's patches exhibited mesenchymal stem cells like properties and promoted inflammatory bowel diseases recovery in mice through IL-22-mediated epithelial repair. Nestin is also widely used as a marker for disease prognosis, including in multiple myeloma, retinal degeneration, and stroke, where it indicates stem cell activity and nerve regeneration. In astrocytoma, Nestin expression has been linked to tumor aggressiveness and is considered a marker for cancer stem-like cells, helping to predict disease progression and potential therapeutic response. Additionally, Nestin plays a role in drug screening higher Nestin expression in ASCs correlates with improved nerve regeneration, and it has been used to evaluate treatments for Alzheimer's and other neurological disorders. Given their broad distribution, Nestin cells may have future applications in disease monitoring, drug testing, and prevention across different tissues. Understanding their functions in development, regeneration, and pathology remains crucial [30].

In Yokota et al., study, patients received the WT1-specific cytotoxic T lymphocyte (CTL) peptide vaccine for a minimum of three months and subsequently demonstrated radiographic and/or clinical signs of tumor progression. Immunohistochemical analysis of paired pre- and post-vaccination tumor specimens confirmed continued expression of the target antigen WT1 in post-treatment tumor cells. Notably, sustained WT1 expression following vaccination was positively associated with both overall survival (OS) and progression-free survival (PFS), indicating that retention of WT1 expression may serve as a prognostic biomarker in vaccine-treated tumors [31].

Vaccination represents a promising immunotherapeutic strategy against Glioblastoma Multiformis, utilizing tumor-associated antigens to elicit a targeted antitumor immune response. This approach aims to activate and sustain adaptive immune surveillance specifically against Glioblastoma Multiformis cells. Currently, four primary vaccine platforms are under investigation for GBM treatment: peptide-based vaccines, DNA vaccines, cell-based vaccines (including dendritic cell vaccines), and mRNA-based vaccines, each offering distinct mechanisms for antigen presentation and immune activation [32].

4. Conclusion

We found that Nestin expression increases with tumor grade and that Nestin was expressed in Astrocytoma. Nonetheless, we might conclude that Nestin is a useful marker to determine the prognosis of an Astrocytoma. The study's findings confirm WT-1's carcinogenic function in Astrocytoma. Differentiating between low- and high-grade Astrocytoma can be aided by WT-1 grading. Thus, WT-1 seems to be a desirable immunohistochemistry marker for use in Astrocytoma in addition to other immunohistochemical markers. Furthermore, WT-1's function in immunotherapy and ability to help select patients for targeted immunotherapy are supported by the fact that Astrocytoma frequently express WT-1.

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Ethics Statement

The project has been approved by the JPMC Ethics Review Board (IRB).

Author contributions

Asma Jalbani: devised the idea and wrote the manuscript. Parisa Bashir: write up, proof reading and tabulation. Prih Bashir: devised the idea, write up and proof reading. Javeria Tunio: editing, statistics and data collection. Farah Siraj: literature search and data collection. Arpana Nihal: literature search and statistical analysis.

Conflict of Interest

There is no conflict of interest among the study's authors.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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