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High-grade Astrocytoma is Associated with Significant Expression of the Wilms Tumor Gene (WT-1) Protein

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ABSTRACT

OBJECTIVE: To investigate the association between different astrocytoma grades and WT-1gene protein immunoexpression at the Pathology Department of a tertiary care hospital.
METHODOLOGY: In this cross-sectional study, sixty biopsies of Astrocytoma were incorporated using non-probability convenience sampling. All cases of various grades of Astrocytoma received in the Department of Pathology, Basic Medical Sciences Institute, JPMC, Karachi, from January 2019 to December 2022, were thoroughly reviewed and included in this research. Biopsy samples with an insufficient amount of material, metastatic Carcinoma and CNS tumors other than Astrocytoma were excluded. Results were analyzed using IBM- SPSS version 23.0. The Pearson Chi-Square test of independence was applied, and P-values less than 0.05 were considered significant.
RESULTS: A total of 60 cases of astrocytomas were immunostained for WT-1. Of the 56 samples, 93.3 percent had positive WT-1 cytoplasmic immunostaining. A statistically significant correlation ($p < 0.001$) was found between the WT-1 score and tumour grade, with low-grade (grades I and II) astrocytomas scoring lower and high-grade (grades III and IV) astrocytomas scoring higher.
CONCLUSION: The research confirms WT-1's role in astrocytoma carcinogenesis and aims to assess its expression across different histological grades. Low expression correlates with lower tumour grades, while high expression indicates higher tumour grades. WT-1 can help distinguish between grades, making it a useful immunohistochemical marker. Its frequent expression in astrocytic tumors supports its potential in immunotherapy, aiding in the identification of candidates for targeted treatment. WT-1 regulates key cellular processes, making it a promising target for improving treatment outcomes in astrocytoma patients.

KEYWORDS: Astrocytoma, Brain tumour, WT-1, Tumor Grade, Wilms Tumor-1, Immunoexpression

INTRODUCTION

The gene that generates nephroblastomas in pediatric children was identified by Max Wilms in 1899 as WT-1 (Wilms' Tumor 1). The genetic makeup and cellular location of WT-1 have been studied concerning several physiological functions, and the amount of research on the protein has significantly increased since then[1].

The Wilms tumor gene has been identified on chromosome 11p13 [2,3]. WT-1 express in code a transcript factor that has a key function in encouraging cell growth and differentiation [4, 5]. Hematologic and solid tumors, such as leukemia, ovarian, breast and brain malignancies, as well as soft tissue sarcomas, including rhabdomyosarcomas and malignant peripheral nerve sheath tumors, have all been correlated with it [6, 7].

Organ development during embryogenesis relies on the coordination of various genes and molecular factors, with the WT-1 gene playing a key role. It produces around 30 isoforms through post-transcriptional changes and regulates tissue maturation. WT-1 functions as a transcriptional regulator through its N-terminal domain, interacting with proteins, RNA, and DNA via its C-terminal domain. It also regulates growth factors, extracellular matrix components, transcription factors, and genes involved in cell growth[8,9]. Addressing WT-1 in the context of glioblastoma (GBM) has led to the investigation of various immunotherapies, including vaccinations, chimeric antigen receptor T-cell (CAR-T) therapy, oncolytic viruses, and immune checkpoint inhibitors[10,11].

The rationale for this study lies in the importance of identifying a reliable correlation between WT-1 and astrocytoma grades to enhance therapeutic and diagnostic strategies. There has been little focused research on the WT-1 gene in relation to astrocytomas, despite its known carcinogenic properties in various malignancies. This study aims to clarify the role of WT-1 in tumor biology, provide insights into its predictive value for tumor behavior, and support its potential as a target for immunotherapy, with the ultimate goal of improving patient outcomes in neuro-oncology. It does this by examining the relationship between WT-1 protein immune-expression and astrocytoma grades.

METHODOLOGY

The current cross-sectional study was conducted at the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, using a non-probability convenience sampling technique.

This study was based on the analytical study of CNS tumor cases diagnosed as Astrocytoma that were received in the Department of Pathology, BMSI, from January 2019 to December 2022. All diagnosed cases of various histological grades of Astrocytoma were subjected to immunohistochemistry. Permission was obtained from the institutional review board (IRB) at JPMC.

All histopathologically confirmed Astrocytoma specimens that had been properly formalin-fixed and paraffin-embedded were included. CNS tumors other than astocytoma, and biopsy samples with an insufficient amount of material were excluded from the studies.

Histopathologic Review

Astrocytoma specimens were preserved in a 10% formalin solution according to established guidelines, and the biopsy sample was then processed and embedded in paraffin. Histopathological sections, 3–4 µm thick, were prepared and stained with hematoxylin and eosin (H&E) to visualize the cells. Two pathologists examined the sections under a light microscope to diagnose the pathology. Patient information, including age, socioeconomic status, and clinical features, was provided in the initial surgical biopsy reports. The fifth edition of the WHO Classification of Tumors of the Central Nervous System is the latest version of the global standard for classifying brain and spinal cord tumors.

Immunohistochemistry

The WT-1 Mouse monoclonal antibody was the primary antibody employed in this investigation. The antibody was purchased from Dako Corporation and was given the catalogue number REF IR055. Using the WT-1 antibody, immunohistochemical staining was performed on 60 selected patient biopsies. Through the use of high-power, low-power, and scanning lenses, every slide was examined using a light microscope. The use of ovarian serous carcinoma ensured a positive control. WT-1 immunoexpression was only deemed positive in neoplastic cells that had cytoplasmic staining. Moreover, astrocytic processes and the fibrillary tumor matrix were shown to express WT-1.

Immuno-histochemical Evaluation

A semi-quantitative scoring arrangement was used to calculate 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 of (0) for no expression, (1) for expression <25%, (2) for expression between 25% and 75%, and (3) for expression >75% was assigned to the frequency of WT-1 expression by cancer cells. For intensity, cancer cells were scored 0 for negative expression or 1 for mild intensity, depending on the strength of WT-1 staining compared to normal glial cells. A score of 2 denoted moderate intensity, while a score of 3 denoted marked notable intensity. Six indices (0, 1, 2, 3, 4, 5, and 6) were produced by summing the frequency and intensity values. The three categories into which these indices fell were marked (indices 5 and 6), moderate (indices 3 and 4), and negative (indices 1 and 2).

Statistical analysis

The data were stored and examined using IBM SPSS version 23.0. For each histological grade, the number of astrocytes was recorded, along with the corresponding percentage. To determine if two categorical variables are related, the Pearson Chi-Square test of independence was employed. Statistical significance was defined as P values < 0.05.

RESULTS

A breakdown of the different Astrocytoma's histological subtypes and grades. The findings show that 16 cases of pilocytic Astrocytoma, or 26.7 per cent of all astrocytoma cases, were all graded as Grade I. Twenty-four cases, or 40 percent of the total, were classified as Grade II diffuse astrocytomas. Six cases, or 10 percent of the total, had anaplastic astrocytoma Grade III. Of the 14 cases of Astrocytoma, glioblastoma multiforme Grade IV was detected in 23.3% of the cases **Table I**.

Table I: The Different Histological Grades of Astrocytoma

Morphological Type	WHO Grade	Overall sum of cases (%)
Pilocytic Astrocytoma	I	16 (26.7)
Diffuse Astrocytoma	II	24 (40)
Anaplastic Astrocytoma	III	6 (10)
Glioblastoma Multiforme	IV	14(23.3)

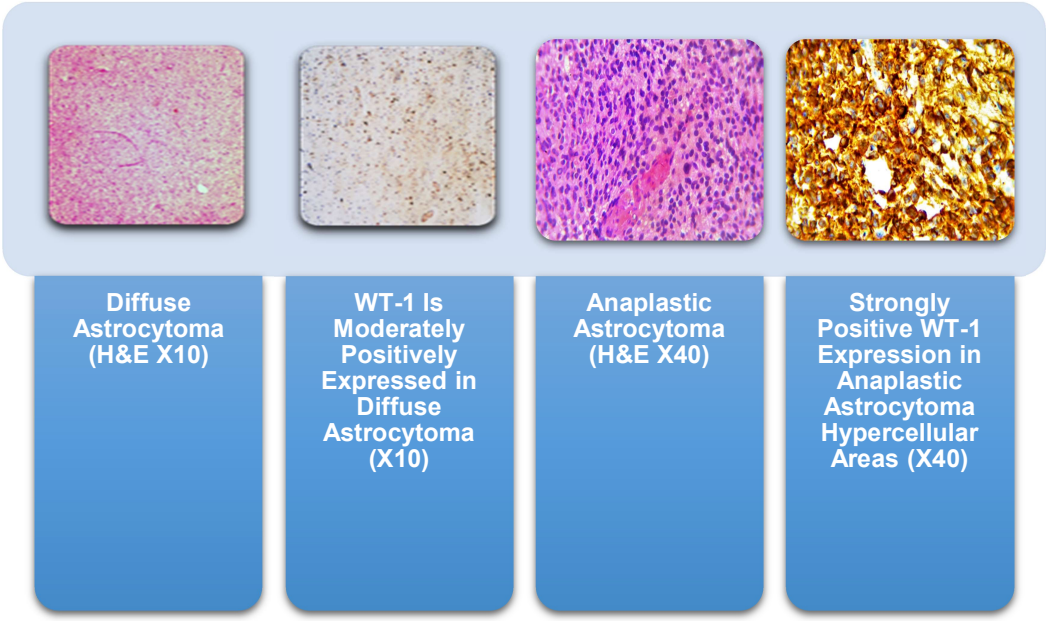
The immunoreactivity for every single case with various types of Astrocytoma is displayed in Table II. WT-1 index values of 1 and 4, respectively, were seen in 15 (25%) and 1 (1.7%) of the grade-I cases (26.7%). Of the forty percent grade-II cases, two (3.3%) had a WT-1 index of 1, two (3.3%) had a WT-1 index of 3, and twenty (33.3%) had a WT-1 index of 4. One case (1.7%) with a WT-1 index of 0 and five cases (8.3%) with a WT-1 index of 6 were among the grade-III patients (10%). One (1.7%) and thirteen (21.7%) of the grade-IV cases (23.3%) had WT-1 indices of 0 and 6, respectively. Immunoreactivity and the grade of Astrocytoma had a significant connection, according to the Pearson chi-square test ($p < 0.001$). **Table II**

Table II: Wilms Tumor-1 Immuno-reactivity in Each Case of Different Astrocytoma Grades

WHO Astrocytoma Grade	Total Cases (%)	WT-1 index=0	WT-1 index.=1	WT-1 index=2	WT-1 index=3	WT-1 index=4	WT-1 index=5	WT-1 index =6	P-value
GRADE-1	16(26.7)	-	-	15(25)	-	1(1.7)	-	-	<0.01*
GRADE-2	24(40)	2(3.3)	-	-	2(3.3)	20 (33.3)	-	-	
GRADE-3	6(10)	1(1.7)	-	-	-	-	-	5 (8.3)	
GRADE-4	14(23.3)	1(1.7)	-	-	-	-	-	13 (21.7)	
OVERALL	60(100)	4(6.7)	-	15(25)	2(3.3)	21(35)	-	18(30)	

* Pearson Chi-Square test was implemented to determine statistical significance ($p<0.05$).

Photomicrograph: I



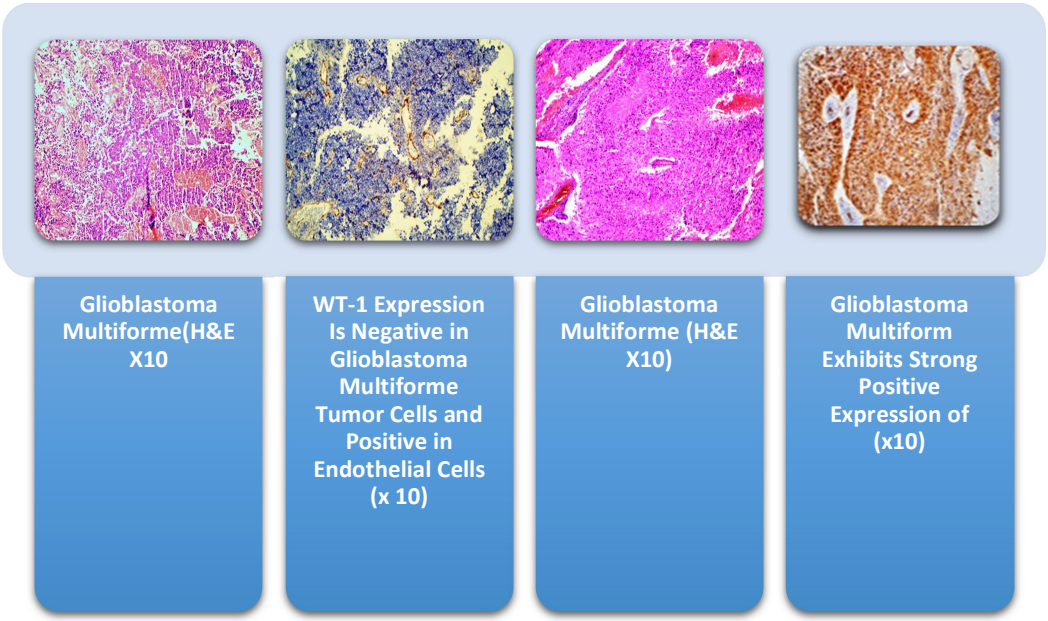
Diffuse Astrocytoma (H&E X10)

WT-1 Is Moderately Positively Expressed in Diffuse Astrocytoma (X10)

Anaplastic Astrocytoma (H&E X40)

Strongly Positive WT-1 Expression in Anaplastic Astrocytoma Hypercellular Areas (X40)

Photomicrograph: II



Glioblastoma Multiforme(H&E X10

WT-1 Expression Is Negative in Glioblastoma Multiforme Tumor Cells and Positive in Endothelial Cells (x 10)

Glioblastoma Multiforme (H&E X10)

Glioblastoma Multiform Exhibits Strong Positive Expression of (x10)

DISCUSSION

The present research project aimed to investigate the immune-histochemical expression of Wilms Tumour-1 protein in various astrocytoma grades. Research has been conducted to determine the function of WT-1 in Astrocytoma in both Western and some Asian countries[12,13]. Regretfully, there hasn't been any attempt to look into WT-1's potential connection to Astrocytoma in Pakistan. We immunostained sixty cases of Astrocytoma with WT-1. Of these, 56(93.3%) had positive cytoplasmic WT-1 immunostaining. WT-1 expression index values were greater in high-grade (III and V) astrocytoma than in low-grade (I and II). The WT-1 score correlated significantly ($p < 0.001$) with astrocytoma grade, showing higher values in high-grade (III and IV) astrocytomas and lower scores in low-grade (I and II) astrocytomas. Considerable correlations were found between WT-1 expressions and Astrocytoma's grade. A WT-1 index of 6 was present in approximately 90% (18/20) of high-grade Astrocytomas. No low-grade astrocytoma was found with a WT-1 index of 6.

Findings of 100%, 91.24%, 95.9%, and 96%, correspondingly, are consistent with the conclusions of several other studies. It was discovered that WT-1 expression was only present in the cytoplasm of cancerous cells[12,14,15]. WT-1 protein was found in the cytoplasm of WT-1 immunopositive tumor cells, as demonstrated in research conducted by **Oji et al. (2004)**, suggesting that WT-1 may have a role other than transcriptional control, potentially in RNA metabolism as a metabolite. **Yokota et al. (2013)** report that substantial cellularity and locations where perivascular proliferation was seen in all positive cases were associated with high levels of WT-1 protein expression. These outcomes align with our findings from the current investigation. Based on the findings of the present study, 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 progression of glial tumors. **Oji et al. (2014)** found that astrocytic tumors with increased WT-1 protein expression levels also exhibited higher tumor grades[14].

Schittenhelm et al. (2009) reported that while 52% of diffuse astrocytomas exhibited positive immunostaining for WT-1, over 75% of high-grade gliomas showed positive expression of WT-1 (score 6) [17].

Bassam et al. (2010) discovered a beneficial association between higher scores and progression to malignancies in Grade I patients, except for one, who had a WT-1 index of 2 with mild intensity[18]. **Yokota et al. (2010)** found the absence of expression in grade II astrocytomas, which is consistent with our results. **Mahzouni and Meghdadi (2012)** found that grade II cases primarily exhibited moderate WT-1 expression, which is consistent with our findings [14,19]. According to **Schittenhelm et al. (2012)**, grade II astrocytomas were reported to exhibit notable expression. However, **Bassam et al. (2014)** found that grade II astrocytomas had a substantial presence of moderate expression, which is in line with the results of the current study[18,19]. The IDH1 mutation in young patients with high-grade Astrocytoma justified the lack of expression in grades III and IV noticed in this investigation. Additionally, **Rauscher et al. (2014)** noted that older patients exhibited higher levels of positive WT-1 expression compared to those with negative WT-1 expression[13]. The current research discovered that every single case of negative WT-1 expression was also noted in youngsters.

Oji et al. (2004) discovered that WT-1 was present in some glioblastoma cell lines yet absent in some[16]. This could explain why WT-1 expression was lacking in a single of the investigated cases. Conversely, **Bassam et al. (2014)** found weak expression in a single glioblastoma case and no negative expression in any of the cases they examined[19]. Both the **Rauscher et al. (2014) and Schittenhelm et al. (2009)** studies found that astrocytomas had considerably higher WT-1 expression levels than oligodendrogliomas. The findings of the present study suggest that WT-1 is involved in the astrocytic differentiation of central

nervous system (CNS) cells, specifically glial cells[13,18]. WT-1 can, therefore, be utilized as a means of diagnosis and to differentiate between astrocytic and oligodendrocytic malignancies. A further investigation found that WT-1 helped differentiate reactive gliosis from tumor recurrence when treatment-related alterations, including necrosis and reactive gliosis, were present[12]. These topics were excluded from the research because the focus was solely on astrocytic tumors. The current investigation revealed a significant correlation between various astrocytoma grades and the overall WT-1 score.

A small number of cases in the current study showed negative WT-1 expression; this could also be the case because specific antibodies are more effective at detecting particular WT-1 isoforms. Different isoforms of this marker should be evaluated independently to confirm isoforms specific to individual lesions. The WT-1 protein, which plays a significant role in regulating tumor genesis and is not expressed in normal brain tissue, emerged as an appealing candidate for immunotherapy against malignancies after it was identified as a tumor-associated antigen. This is especially true for tumors of the brain and spinal cord.

CONCLUSION

The research confirms WT-1's carcinogenic role in astrocytomas and aims to understand its expression across different histological grades better. The quantity and intensity of positively stained cells should be considered when interpreting the results - low expression indicates a low tumor grade, while high expression suggests a high-grade tumor. The WT-1 evaluation can aid in distinguishing between low- and high-grade astrocytomas, making it a valuable immune-histochemical marker in conjunction with others. Additionally, the frequent expression of WT-1 in astrocytic tumors supports its potential use in immunotherapy, helping to identify candidates for targeted treatment. Its role in regulating key cellular processes such as growth and differentiation makes WT-1 a promising target for immune-based therapies, potentially improving treatment outcomes for astrocytoma patients.

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AUTHOR CONTRIBUTION

Jalbani A: Devised the idea and wrote the manuscript
Rahat N: Study final approval
Shahzad H: Editing, statistics and data collection
Bashir P: Editing, statistics and data collection
Zulfiqar F: Editing, statistics and data collection
Momin Z: Editing, statistics and data collection

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