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Role of *Wilms' tumor 1* in diagnosis and grading of astrocytomas

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ABSTRACT

Background: *Wilms' tumor 1* (*WT1*) mutation has recently been detected in gliomas. Growing data indicate that *WT1* mutation plays a causal role in gliomagenesis and is overexpressed in most glioblastomas. Emerging immunotherapy targeting *WT1* has shown to be effective in resistant glioblastomas in clinical trials. *WT1* expression and its potential utility in various grades of astrocytomas is still unclear and needs further elucidation. The evaluation of *WT1* can be done by molecular or immunohistochemical methods. As immunohistochemistry is easier with wider routine use, immunoexpression of this biomarker was studied. **Aims and Objectives:** This study aimed to evaluate *WT1* immunoexpression across different histological grades of astrocytomas and differentiate low-grade astrocytomas from reactive astrogliosis. **Materials and Methods:** This was an observational prospective study on 54 cases of astrocytomas. **Results:** In the present study a total of 54 cases have been diagnosed as astrocytomas. Grade IV was the most common (31%) followed by grade II (44%). *WT1* score correlated with histological tumor grades ($P<0.001$) with a higher score in a higher grade. It was also observed that different tumor grades depicted two distinct expression patterns. *WT1* score and pattern were valuable in differentiating high- and low-grade astrocytomas. **Conclusion:** This study supports the oncogenic role of *WT1* in astrocytomas. *WT1* was found to be valuable in distinguishing different grades of astrocytomas. The scoring and distinctive patterns of *WT1* can aid in distinguishing high-grade and low-grade astrocytomas. However, one needs to be careful in pilocytic astrocytomas in which there is a mixed expression. *WT1*, therefore, appears to be an attractive immunohistochemistry (IHC) marker to be used concomitantly with other IHC markers in astrocytomas. In addition, the frequent expression of *WT1* in astrocytomas supports its promising role in immunotherapy and its potential to guide patient selection for targeted immunotherapy.

Key words: Astrocytomas; Gliomas; Immunohistochemistry; *Wilms' tumor 1*

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INTRODUCTION

In India, there are between 5 and 10 central nervous system (CNS) tumors/100,000 people, with an upward tendency. The most frequent primary brain tumors are astrocytomas (38.7%), with high-grade gliomas (59.5%) constituting the majority.[1] Tumors of the central nervous system are distinctive in that they have a few distinctive characteristics. In contrast to other locations, benign tumors may have the capacity to endanger life.[2] Over 75% of glial tumors, which make up 42% of all primary CNS neoplasms, are malignant.[3] The biggest obstacle in oncology is still managing these tumors. Human gliomas are classified histopathologically and graded for malignancy using standards set forth by the World Health Organization (WHO).[4] These criteria, however, are constrained by subjective interpretations, leading to inter- and intraobserver heterogeneity.[5] Mitotic counting is a key component in the grading of these tumors since proliferation is a fundamental mechanism in the creation of gliomas. Glioma grading is inaccurate and may negatively affect therapy, prognosis, and follow-up because it can be challenging to identify and quantify mitotic figures in hematoxylin and eosin-stained (H&E) sections. The diagnosis of several neurological illnesses has been significantly altered

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by immunohistochemistry (IHC) employing monoclonal or polyclonal antibodies. This method makes it possible to accurately characterize the presence of the distinctive antigen in a sensitive and repeatable manner, improving the diagnostic tool for CNS tumors.[6]

A transcriptional factor that is critical for stimulating cell development and differentiation is encoded by *Wilms' tumor 1* (*WT1*).[7] Numerous cancers have been linked to its function. *Wilms' tumor* was where *WT1* was initially discovered.[8] Numerous cancers have been linked to its function. It was later discovered to be overexpressed in several solid tumors and leukemias, including ovarian and breast cancers.[9] Recent research has demonstrated its function in gliomas.[10,11] Different histological grades of astrocytomas have been associated with variable rates of *WT1* overexpression.[12] However, there are still disagreements about its potential and insufficient evidence to support its value. *WT1* mutations can be found using an effective and less labor-intensive method called IHC. However, more research has to be done on the immune characteristics of *WT1* across different grades. *WT1* may be a potential target for immunotherapy in high-grade gliomas, according to recent clinical studies of cancer immunotherapy that focus on this protein, particularly in situations where the tumor is resistant to treatment.[12,13] Before starting therapy, it is necessary to screen for the existence of the *WT1* mutation, which can help with clinical choices. Understanding *WT1*'s full function in astrocytomas may aid in diagnostics and provide new opportunities for a potential target for cancer therapy.[14] We investigated *WT1* by IHC in astrocytomas to assess its diagnostic value.

Aims and objectives

This study aimed to evaluate *WT1* immunoexpression across different histological grades of astrocytomas differentiate low-grade astrocytomas from reactive astrogliosis.

MATERIALS AND METHODS

Study design

This is a prospective and retrospective study for 18 months (January 2019–June 2021) to be conducted at Osmania General Hospital, Hyderabad.

Study population

A total of 54 cases are taken up for study.

Inclusion criteria

All neurosurgically excised specimens were diagnosed as astrocytomas on histomorphology.

Exclusion criteria

Leftover squash specimens and mixed neuronal-glial tumors.

Examination of cases

Due importance was given to recording a brief clinical history with age, inpatient registration number, biopsy number, presenting symptoms and signs, and CT and MRI findings. The specimens were received in 10% formalin. Measurements of the specimens were recorded and thorough gross examination was carried out and salient features such as hemorrhage, necrosis, and calcification were recorded. Depending on the volume of the tumor, an adequate number of blocks were given. After routine tissue processing, H&E staining was done and slides were microscopically examined.

Histopathological examination

The surgical specimens were fixed in 10% buffered neutral formalin and processed for paraffin embedding. For histopathological examination, 3-μm-thick sections were stained with H&E. Histological diagnosis and grading were done according to the WHO classification of central nervous system tumors.[15,16] based on cellularity, pleomorphism, number of mitoses per 10 high-power field (HPF), presence or absence of microvascular proliferation, and necrosis.

IHC

For IHC analysis, additional sections were prepared for conventional IHC with *WT1* antibody (clone 6F-H2 mouse monoclonal anti-human antibody-Dako) on all selected cases. Ki-67 (MIB-1, Dako, prediluted), according to the standard procedures on automated immunostainer BioGenex X Matrix (49026 Milmont Drive, Fremont, CA 94538, USA).

Immunohistochemical assessment

All cases were studied for histomorphology and IHC by experienced pathologists independently. Brown stain in the cytoplasm is considered positive. In the endothelium of the capillaries present in the examined sections (positive internal controls), consistent staining of cytoplasm was noted. Tumor cells with positive cytoplasmic staining were considered positive. Cytoplasmic staining of neoplastic cells showing moderate-to-high intensity was considered positive. Weak or equivocal staining was excluded.

Evaluation of staining: *WT1* expression was evaluated using a semi-quantitative scoring method and scored as 0 (no staining), 1 (singular positive cells, ≤1%), 2 (>1–25%), 3 (26–50%), and 4 (>50%), which was comparable to that proposed earlier by Manocha and Jain.[17] For counting the immunopositive cells, 10 HPFs (×40) were selected and systematically randomized throughout the section. The correlation of *WT1* expression and histological grade was calculated.

Statistical analysis

Descriptive statistics were analyzed with SPSS version 17.0 software. Continuous variables are presented as mean (min–max). Categorical variables are expressed as frequencies and percentages. The Pearson Chi-square test or the Chi-square test of association was used to determine if there is a relationship between two categorical variables. Probability ($P<0.05$) were considered statistically significant.

RESULTS

The spectrum and demographic details of astrocytomas are shown in Table 1.

Characteristics of patients

There was a male predominance (males: 59.30%; females: 40.70%). In the present study, a total of 54 cases have been diagnosed as Astrocytomas (10 cases of pilocytic astrocytomas, 20 cases of diffuse astrocytomas, 4 cases of gemistocytic astrocytomas, 3 cases of anaplastic astrocytomas, 17 cases of glioblastomas,) with a male: female ratio of 1.45:1. The mean age was 41.86 years with the age range of 5–75 years. Grade IV was the most common (31%) followed by grade II (44%).

WT1 immunohistochemical expression

WT1 positivity was detected in all astrocytomas (54/54; 100%). Positivity was detected in the cytoplasm, astrocytic processes, and fibrillary tumor matrix. Immunostaining in the endothelial cells served as the positive internal control for *WT1*. *WT1* was positive in neoplastic areas, whereas adjacent normal glial tissues were negative. All cases of reactive gliosis in the control group were negative for *WT1*.

Table 1: Summary of demographic details and spectrum of astrocytomas

Characteristics	No. of patients (n, %)
Sex	
Male	32 (59.30)
Female	22 (40.70)
Age distribution	
<15 years	14 (26)
16–30 years	10 (18)
31–45 years	15 (28)
46–60 years	07 (13)
>60 years	08 (15)
Distribution of astrocytomas	
Pilocytic astrocytoma	10 (19)
Gemistocytic astrocytoma	4 (7)
Diffuse astrocytoma	20 (37)
Anaplastic astrocytoma	3 (6)
Glioblastoma	17 (31)
Grades of astrocytomas	
Grade-I	10 (19)
Grade-II	24 (44)
Grade-III	3 (6)
Grade-IV	17 (31)

Table 2 summarizes the score and pattern of *WT1* expression in different grades of astrocytomas. The immunostaining proportion score varied from 2 to 4. None of the cases showed a score of 1. Higher expression was found in more cellular areas. In tumors showing heterogeneity, the *WT1* score was higher in high-grade areas compared to low-grade areas. Staining was absent in necrotic areas. High *WT1* expression of score 4 was seen in a majority of glioblastomas (17/54; 31%) and anaplastic astrocytomas (3/54; 6%).

Combining grade III and IV as high-grade astrocytomas and grade II and I as low-grade astrocytomas, high-grade astrocytomas, and low-grade astrocytomas was found statistically significant in differentiating these two categories ($P<0.001$).

Pilocytic astrocytoma with *WT1* expression (Figure 1a and b) and diffuse astrocytoma with *WT1* expression shows in Figure 1c and d.

DISCUSSION

The most frequent gliomas, accounting for <60% of all brain tumors, are astrocytomas.[18] *WT1*, a newly discovered molecular marker in gliomas, has been linked to gliomagenesis.[19] The development of *Wilms' tumor*, a juvenile renal neoplasm, featured frequent deletions at the chromosome 11p13 region, leading to the discovery of *WT1* as a tumor suppressor gene.[20] A zinc finger transcriptional factor involved in cell proliferation and differentiation is encoded by the *WT1* gene. Recently, melanomas, various solid tumors, and numerous hematological malignancies have been linked to *WT1*'s oncogenic function.[21]

A few studies have revealed that *WT1* expression is frequently seen in gliomas and plays a part in the development of malignant astrocytic tumors.[22] The high prevalence of *WT1* in gliomas and the lack of *WT1* in healthy astrocytes imply that WT1 has an oncogenic function and is an important marker for gliomas.[23] In addition, glioblastoma clinical studies of targeted immunotherapy targeting the *WT1* protein have yielded encouraging outcomes. According to research, transitory *WT1* silencing makes tumors more responsive to chemotherapy and radiation.[24] indicating that *WT1* may be a good candidate for immunotherapy in aggressive high-grade gliomas. Therefore, assessing *WT1* is crucial for neuropathologists and oncologists to forecast the effectiveness of anti-*WT1* treatment. The *WT1* mutation assessment approach using IHC seems to be more practical for everyday use. There are still questions about

Table 2: Correlation of percentage positivity (score) and patterns of *WT1* with different histological grades in astrocytomas

	Grade-I (n=10) (%)	Grade-II (n=24) (%)	Grade-III (n=3) (%)	Grade-IV (n=17) (%)	P-value
WT1 score*					
Score-1	0	0	0	0	<0.0001
Score-2	3 (30)	1(4.2)	0	0	
Score-3	5 (50)	21 (87.5)	0	3 (17.6)	
Score-4	2 (20)	2 (8.3)	3 (100)	14 (82.4)	
WT1 pattern**					
Dense	0	0	2 (67)	14 (82.4)	<0.0001
Loose	6 (60)	22 (91.7)	0	0	
Loose and dense	4 (40)	2 (8.3)	1 (33)	3 (17.6)	

**WT1* score and ***WT1* pattern showed significant correlation (P<0.001) with histological tumor grade.WT1: *Wilms' tumor 1*

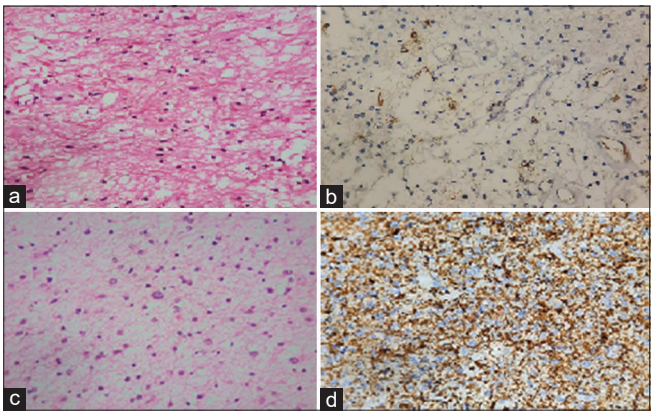

Figure 1: (a) H and E (×40) Rosenthal fibres; (b) (immunohistochemistry) *Wilms' tumor 1* positive; (c) and (d) diffuse astrocytoma with *WT1* expression.

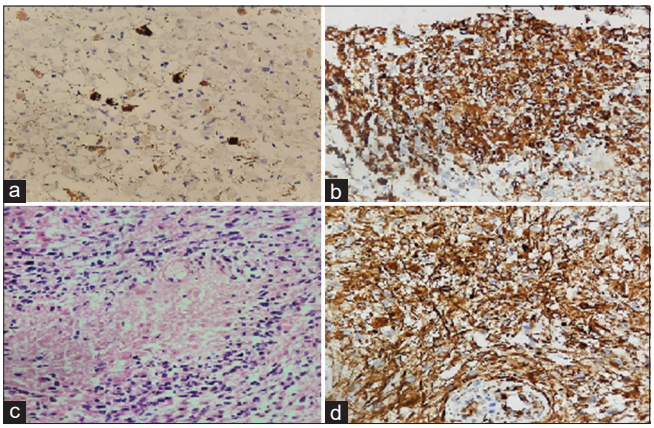

Figure 2: (a) Gemistocytic astrocytoma with GFAP expression-(immunohistochemistry, IHC) *Wilms' tumor 1* (WT1) Positive; (b) Anaplastic astrocytoma with WT1 expression-(IHC) WT1 positive; (c) Glioblastoma with characteristic histological features-H&E (×40) palisading necrosis; (d) Glioblastoma with WT1 expression (IHC) WT1 positive

the potential of *WT1* immunoexpression, and its use and validity have not been fully established. Therefore, more research is required to clarify the IHC technique and the potential significance of *WT1* in astrocytomas (Figure 2).

In the current investigation, *WT1* expression was identified in each of the 54 astrocytomas (100%) that were examined. Similar findings from earlier trials with positive ranging between 80.9% and 100%[25,26] have been reported. Although the majority of *WT1* molecular investigations have revealed 100% positivity,[26] several research employing the IHC technique have discovered missing expression in a small number of astrocytomas. In their analysis of 73 astrocytomas, Mahzouni and Meghdadi[27] observed that 97.3% of the time, *WT1* protein was expressed. The two exceptions were a glioblastoma and a pilocytic astrocytoma. *WT1* expression varied in our study's astrocytomas, but none of them tested negative for it. In addition, Clark et al.,[28] had noted that normal glial cells did not contain *WT1* protein.

We found a statistically significant correlation between *WT1* expression and WHO histological tumor grade in astrocytomas. Higher expression of *WT1* was seen in higher tumor grades supporting its role in tumorigenesis and tumor progression. It can, therefore, be suggested that higher expression favors a higher grade of tumor and can be useful in differentiating high and low-grade Astrocytomas when dealing with challenging biopsies. Hashiba et al.,[29] observed that astrocytomas showed strong expression of *WT1* protein in high-grade astrocytomas, especially in glioblastoma with *WT1* expression score of 3 or 4 in 31 of 34 cases (91.2%) and the expression decreased in frequency in the lower grades. Consistent with our results, Mahzouni and Meghdadi[27] found that high *WT1* expression was significantly more in glioblastoma as compared to low-grade astrocytomas. Clark et al.,[28] suggested that *WT1* aids to maintain the high proliferative rate in glioblastoma. They found that downregulation of *WT1* caused reduced tumorigenicity of the *in vitro* as well as *in vivo* glioblastoma cell line; hence, *WT1* can serve as a promising target for new molecular glioblastoma therapies.

In the literature, *WT1* expression in astrocytomas is described as cytoplasmic staining in the delicate cell processes as opposed to nuclear immunostaining in *Wilms' tumors*. This is elucidated by the ability of WT1mprotein to transport from the nucleus to the cytoplasm. Although *WT1* immunoexpression in gliomas has been studied, the pattern of *WT1* protein expression has not been described. Hashiba

et al., observed *WT1* as a speckled and heterogeneous pattern in many cases contrasting to a homogeneous appearance in a few of them, but its significance was not further highlighted.[8,30] In this study, we observed two types of *WT1* patterns of dense and loose types in different grades of astrocytomas. A dense pattern diffusely or focally was seen in a majority of high-grade astrocytomas suggesting that a dense pattern is indicative of high-grade astrocytomas. A loose pattern was frequently encountered in low-grade astrocytomas. The different staining patterns of *WT1* may help in challenging the distinction of these entities. Some of the pilocytic astrocytomas (also showed a focal dense pattern with biphasic loose and dense areas.

The most logical explanation for this phenomenon is that its biphasic pattern is a result of the biphasic histology that distinguishes pilocytic astrocytomas from other types of astrocytomas. One must exercise caution while dealing with pilocytic astrocytomas since they can have both a dense pattern and a focal high expression score. In pilocytic astrocytomas, interpretation should be done in conjunction with distinctive histomorphology findings and the Ki-67 index. Additionally, we noticed a *WT1* score and pattern association that was statistically significant ($P<0.001$). Our findings imply that the interpretation of *WT1* should take into account both the percentage score and the staining pattern. A high-grade astrocytoma is indicated by a high score and dense pattern, and vice versa. The cell density of *WT1*-expressing glioma cells, which is frequently correlated with the WHO grade of glioma, is the most likely cause of the distinctive staining pattern of loose or dense patterns. Astrocytoma proliferation as measured by the monoclonal antibody Ki-67/MIB-1 is a well-established technique for determining the biology and prognosis of tumors. In this study, there was a positive connection between *WT1* expression and Ki-67, with greater *WT1* expression indicating higher Ki-67. Were noticeably fewer *WT*1-positive instances in recurring tumors. However, they have not mentioned any modifications connected to the therapy.

Limitations of the study

The limitation of diagnostic role of W1 and expression in different histological grades. The progression of the disease was not assessed in the study since follow-up was difficult.

CONCLUSION

Our results support the diagnostic role of *WT1* in astrocytomas. We have attempted to further specify the WT1 expression in different histological grades of astrocytomas. *WT1* expression significantly correlated with WHO histological tumor grade and Ki-67 index.

It is suggested that interpretation should be based on both score and pattern. Higher expression scores and dense patterns favor higher tumor grade, whereas the lower expression and the loose pattern are suggestive of lower tumor grade. The scoring and distinctive patterns of *WT1* can aid in distinguishing high-grade and low-grade astrocytomas. However, one needs to be careful in pilocytic astrocytomas in which there is a mixed expression.

WT1, therefore, appears to be an attractive IHC marker to be used concomitantly with other IHC markers in astrocytomas. In addition, the frequent expression of *WT1* in astrocytomas supports its promising role in immunotherapy and its potential to guide patient selection for targeted immunotherapy.

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JERS- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article; **BN-** Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **BN, MR-** Design of study, statistical analysis and interpretation; **JERS -** Review manuscript; **BN, NB -** Review manuscript; **MR-** Literature survey and preparation of figures; **MR -** Coordination and manuscript revision.

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