Immunoexpression of WT1 in Astrocytoma and its Correlation with Histopathological Grade

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Abstract

Background: Astrocytoma comprises a group of neoplasms that differ in their location in the Central nervous system, morphologic features and invasive behavior. These are therapeutically challenging for their infiltrative growth pattern, occasionally resistant to conventional therapies and invariably high grade tumors have shown poor prognosis. Moreover biological behavior of astrocytoma and chance of recurrence cannot be ruled out by histopathological evaluation alone. So, new predictive marker for determining tumor progression is at stake.

Objectives: The purpose of the study was to evaluate WT1 expression in astrocytoma and its correlation with histological grade.

Methods: The cross-sectional observational study was conducted in the Department of Pathology, Dhaka Medical College (DMC) from September 2019 to August 2021. Histologically diagnosed 51 cases of different grades of astrocytoma were included in this study. Immunostaining with WT1 protein was done in all cases. The data were collected and statistical analysis was done by SPSS.

Results: The mean age for grade I, II, III and IV astrocytoma's are 14, 27.5, 38.6 and 44 years respectively. There were 30 male and 21 female patients with the ratio 1.42:1. Among 51 cases of astrocytoma's Grade I, II ,III and IV are 10, 15, 8 and 18 in number. By Immunohistochemical study positive WT1 expression was seen in all cases of astrocytoma's (100%). Out of 25 cases of low grade astrocytoma's (WHO grade I and II), expression was found mild in 12 (48%) cases, moderate in 11 (54%) cases and marked only 02 (8%) cases. Regarding 26 cases of high grade astrocytoma's (WHO grade III and IV) expression found mostly marked in 16 (61.5%) cases, moderate in 09 (34.7%) and mild only in 01 (3.8%) cases. These data shows WT1 expression increases with WHO tumor grades and significant positive ($p<0.001$) correlation between WT1 expression and tumor grade.

Conclusion: The study reveals positive correlation between WT1 expressions with WHO tumor grade in astrocytoma. Furthermore, evaluation of WT1 with histopathological grading may provide information about tumor progression as well as guide the clinicians for therapeutic purposes.

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Keywords: Astrocytoma, tumor grade, WT1 expression, tumor progression, Glioblastoma

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Introduction

Astrocytoma are the most common primary intracranial neoplasms arising from glial tissue-supportive cells of brain. The annual incidence of primary brain tumors is approximately 22 per 100000 population.[1] Among all tumors, brain tumor is the 22nd most common tumor Worldwide. Gliomas represent 81% of malignant brain tumors of CNS diagnosed in the United States. Among these, astrocytoma contribute 75% of all gliomas.[2]

WHO classified astrocytic brain tumor into four grades on the basis of cellularity, nuclear atypia, mitoses, necrosis and microvascular proliferation (MVP)-grade I (pilocytic astrocytoma), grade II (diffuse fibrillary astrocytoma), grade III (anaplastic astrocytoma) and grade IV Glioblastoma. Among the histological grades, diffuse fibrillary astrocytomas represent 10-25%, anaplastic astrocytomas approximately 10% and glioblastomas between 75-85% of all astrocytic neoplasms.[3,4]

Although surgical resection is treatment of choice, due to their infiltrative nature grades II-IV are not cured by surgical resection alone and the survival rate has not greatly changed over time.[5] Despite the synergistic multimodal strategies all these treatment is of limited utility and patients with GBM have a poor prognosis, with a progression-free survival of 7-8 months, a median survival of 14-16 months and 5-year overall survival. The clinical challenge with the astrocytic neoplasm is location near inoperable area that limits complete surgical resection of the tumor.[6] Moreover, biological behavior of astrocytoma and chance of recurrence cannot be ruled out by histopathological evaluation alone. So, new predictive marker for determining tumor progression as well as prognostic marker for better outcome is needed.

The WT1 gene encodes a zinc finger transcription factor, functions as activator or repressor for many growth factor genes. Wild type WT1 has oncogenic properties and showed overexpression in astrocytic tumors.[7] Several studied demonstrated that expression of WT1 in primary astrocytic tumors increases with malignancy . Furthermore, increased WT1 expression in astrocytoma were associated with a worse prognosis and unfavorable outcome.[8,9]

Clinical trials has been going with WT1 as a novel antigen for cancer immunotherapy. Researches are ongoing targeting WT1 protein, which have shown promising results in glioblastomas chiefly in resistant cases, suggesting that WT1 can be a possible target for immunotherapy in high-grade gliomas.[10] Therefore, Anti-WT1 strategies may prove useful in improving the response of glioblastoma to radiotherapy, thus potentially improving patient's survival.

The present study was conducted to evaluate the expression of WT1 in Astrocytic brain tumors in accordance with its histopathological grade according to the recommendations of WHO among Bangladeshi patients.

Methods

This study was conducted after receiving approval from ethical committee of Dhaka Medical College. This is a cross-sectional observational study with a purposive sampling done over a period of two years from September 2019 to August 2021. Histologically diagnosed Astrocytoma cases at the Department of Pathology, Dhaka medical college, Dhaka were collected. A total of 51 cases were included in this study.

Patient histologically diagnosed as a case of astrocytoma irrespective of age and sex were included but patient receiving chemo and radiotherapy were excluded from this study.

Detailed clinical information were obtained by taking history and recorded in clinical proforma. Corresponding slides and paraffin blocks were collected. Representative sections from each paraffin block (paraffin blocks with maximum tumor bulk were chosen) and subsequently IHC stain with WT1 was done. The histologic findings such as cellularity mitotic activity, microvascular proliferation (MVP) and necrosis were reevaluated. Formalin fixed paraffin embedded tissue sections were stained with WT1 antibody using standard protocol compatible for DAKO Envision FLEX+ detection system of immunohistochemistry.

Scoring system

Evaluation of immunostaining and scoring was performed by light microscopy. All slides were examined for positively stained tumor cell cytoplasm regardless of tumor grade. Brownish cytoplasmic staining of tumor cells was considered positive. The expression of WT1 were analyzed in ten microscopic fields. The mean expression of ten microscopic fields has been evaluated. The WT1 index score and expression was calculated. Then information was recorded in a prepared proforma.

The percentage of staining was scored as follows:
0=0%. 1= <25%of tumor cells; 2= 25-75% of tumor cells; 3= >75% of tumor cells
The intensity was scored as follows:
0= Negative; 1= Mild; 2= Moderate; 3= Marked

WT1 index was determined as the sum of frequency and intensity scores giving us six indices (0, 1, 2, 3, 4, 5 and 6). The six indices were grouped as, negative (0 index), mild (1 and 2 indices), moderate (3 and 4 indices), marked (5 and 6 indices).[11,12]

Statistical analysis

All the recorded data were analyzed SPSS Windows (SPSS Inc., Chicago, Illinois, USA) version 24.0 software. Continuous variables were expressed as mean values (±SD), and categorical variables were expressed as absolute frequency and percentage, which were presented in tables. *P*-value ≤0.05 was considered statistically significant.

Results

Age distribution of study population shows that, astrocytoma's was more common in 3rd decade. 23.5% astrocytoma occur in the age group of 31-40 years. Whereas, it was least common in the age group ≤ 10 years and 61-70 years. (Figure 1)

Out of 51 cases, astrocytomas were more common in male 30 (58.8%) than in female 21 (41.2%). The male female ratio was 1.42:1 (Figure 2)

The distribution of the study patient by tumor location. It was observed that astrocytoma was widely distributed throughout the CNS. Majority of the lesions were at the temporal region accounting 7 out of 51 patient (13.7%) followed by parietal lobe 6 (11.8%).(Table I)
The mean (SD) age of grade-I, II, III and IV was 14.55±8.55, 27.53±9.36 38.63±8.57 and 44.94±14.57 years respectively. The age differences in different grades were found statistically significant ($p < 0.05$). (Table II)

Table III shows the association between grading with tumor location. It was observed that almost half (50.0%) patients had others tumor location in grade I, 7(46.7%) in grade II, 4(50.0%) in grade III and 10(55.5%) in grade IV. The difference was not statistically significant ($p>0.05$) among four groups.

It was observed that out of 51 patients, 13(25.4%) cases had mild WT1 expression, 20(46.9%) had moderate and 18 (38.8%)

patients had marked WT1expression (Figure 3)

Table V shows WTI expression in four grades of astrocytoma in the study population. Out of 51 cases, grade I, II, III and IV are 10, 15, 8 and 18 in number. In these 10 cases of grade I showed WT1 expression as mild in 06(46.2%), moderate 03(15.0%) and marked only 01(5.6%) cases. In grade II out of 15 cases expression was mild in 06(46.2%), moderate 08(40.0%) and marked only 01(5.6%) case. Regarding grade III out of 8 cases, expression was moderate in 03(15.0%) and marked 05(27.7%). Among 18 cases of grade IV, marked in 11(61.1%), moderate 06(30.0%) and mild only 01(5.6%) cases It was observed that WTI expression increases with WHO tumor grade. The difference was statistically significant.

It was observed that almost 8 (61.5%) cases with less prominent MVP showed mild WT1 expression, whereas 10 (55.6%) cases with prominent MVP showed marked WT1 expression. The difference was statistically significant ($p<0.05$) between three groups. (Table VI)

The Spearman's correlation test between WT1 Expression and tumor grade.WT1 expression categorized into mild, moderate and marked. It was observed that out of 25 cases of low grade (Grade I and II), expression was found mild in 12(48%), moderate in 11(44.0%) and marked in only 02(8%) cases. Regarding 26 cases of high grade astrocytomas (Grade III and IV), expression was found mostly marked in 16(61.5%), moderate in 9(34.6%) and mild in only 01(3.4%)cases. The value of Spearman's correlation coefficient was 0.632, which is positive significant ($p<0.001$).(Table VII).

The value of Spearman's correlation coefficient was 0.632, which is positive significant ($p<0.05$) correlation. Therefore, there was positive correlation between WT1 Expression and tumor grade (Figure 4)

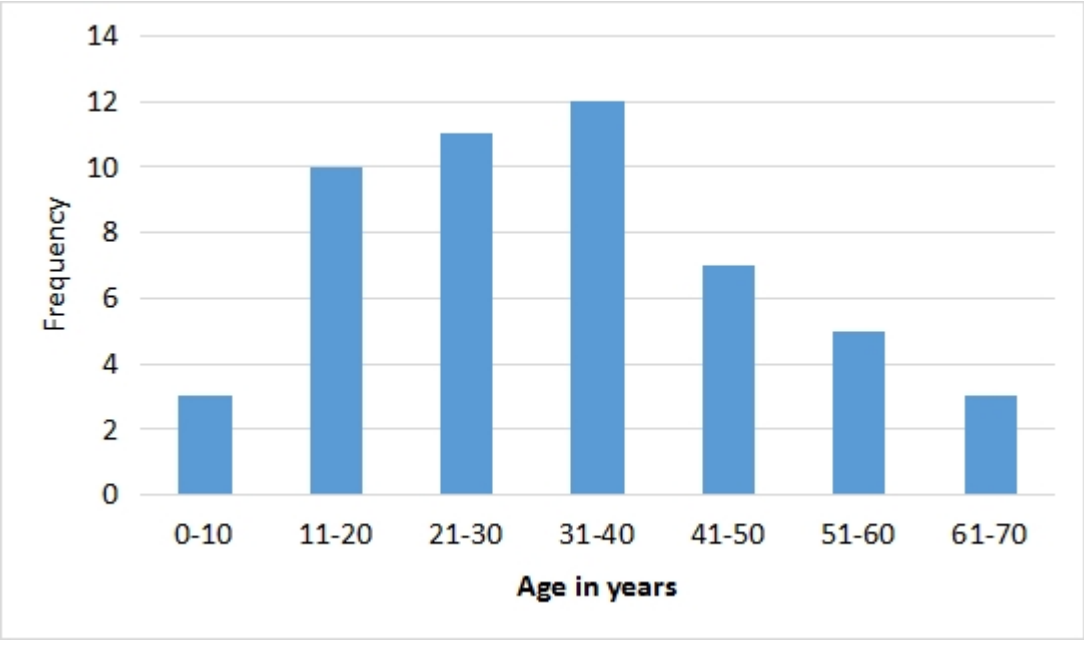


Figure 1. Bar diagram of age distribution of study population (n=51)

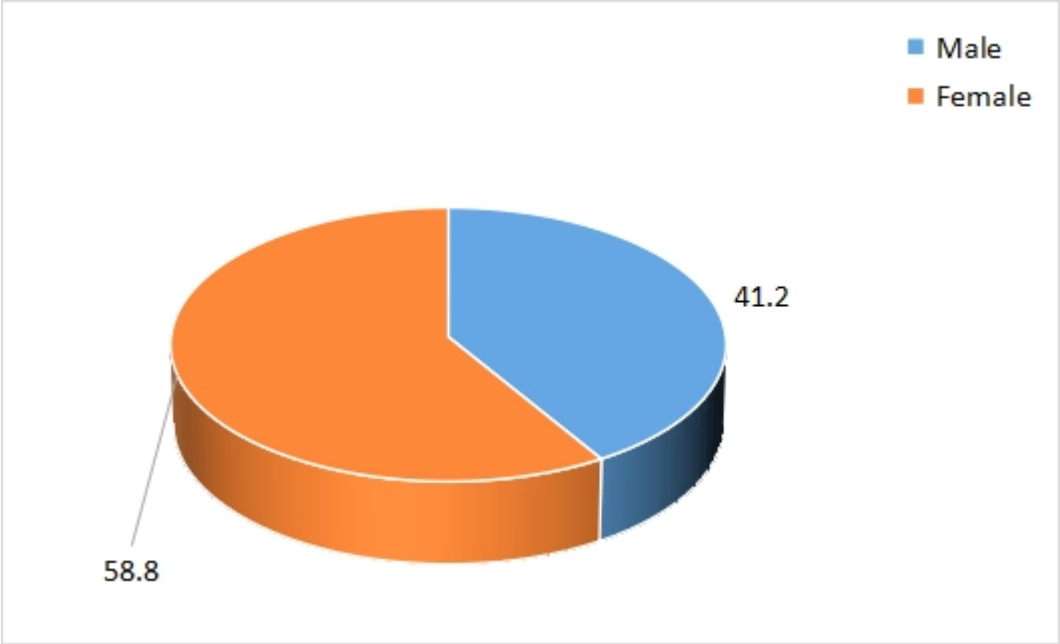


Figure 2. Pie chart shows distribution of study population according to sex.

Table I: Distribution of the cases according location of tumor (n=51)

Tumor location	Number of patients	Percentage
Frontal	4	7.8
Parietal	6	11.8
Temporal	7	13.7
Fronto-parietal	2	3.9
Temporo-parietal	4	7.8
Occiptal	3	5.9
Thalamus	5	9.8
Cerebellar	2	3.9
Posterior fossa	5	9.8
Spinal cord	1	2.0
Unknown	5	9.8
Others (ventricular SOL, suprasellar, corpus callosum)	6	11.8

Table II: Distribution of histopathological grade according to age (n=51)

Grade	Mean ± SD	Range (min-max)	P value
I	14.55±8.55	2.5,35	0.001s
II	27.53±9.36	13,45	
III	38.63±8.57	27,52	
IV	44.94±14.57	13,65	

s = Significant

ANOVA test was done to measure the level of significance.

Table III: Distribution of histopathological grade according location of tumor(n=51)

Tumor location	Grade								P value
	Grade I (n=10)		Grade II (n=15)		Grade III (n=8)		Grade IV (n=18)		
	n	%	n	%	n	%	n	%	
Frontal	3	30.0	0	0.0	0	0.0	1	5.6	0.354[ns]
Temporal	1	10.0	4	26.7	1	12.5	1	5.6	
Parietal	1	10.0	3	20.0	0	0.0	2	11.1	
Occiptal	0	0.0	1	6.6	2	25.0	0	0.0	
Thalamus	0	0.0	0	0.0	1	12.5	4	22.2	
Others	5	50.0	7	46.7	4	50.0	10	55.5	

ns= not significant

*Fisher Exact test was done to measure the level of significance.

Table V: Association of histological diagnosis with WHO grade and WT1 Expression (n=51)

Diagnosis	Grade	WT1 Expression						P value
		Mild (n=13)		Moderate (n=20)		Marked (n=18)		
		n	%	n	%	n	%	
Pilocytic astrocytoma	Grade-I	6	46.2	3	15.0	1	5.6	0.001[s]
Diffuse Fibrillary astrocytoma, NOS	Grade-II	6	46.2	8	40.0	1	5.6	
Anaplastic astrocytoma, NOS	Grade-III	0	0.0	3	15.0	5	27.7	
Glioblastoma multiforme, NOS	Grade-IV	1	7.6	6	30.0	11	61.1	

s= significant

*Fisher Exact test was done to measure the level of significance.

Table VII: Association of microvascular proliferation and WT1 Expression

Microvascular proliferation	WT1 Expression						P value
	Mild (n=13)		Moderate (n=20)		Marked (n=18)		
	n	%	n	%	n	%	
Less prominent	8	61.5	13	65	6	33.3	0.033[s]
Moderately prominent	4	30.8	2	10	2	11.1	
Prominent	1	7.7	5	25	10	55.6	

s= significant

p value reached from Chi-square test

Table VII: Spearman's correlation test between WT1 Expression and tumor grade

Histological (Rx) Grade	WT1 Expression						Correlation coefficient	P value
	Mild (n=13)		Moderate (n=20)		Marked (n=18)			
	n	%	n	%	n	%		
Low grade (I and II)	12	48	11	44	2	8	r=0.632	0.001
High grade (III and IV)	1	61.5	9	34.6	16	3.4		

r = 0.632, which is positive significant.

*r value reached from Spearman's rank correlation coefficient test

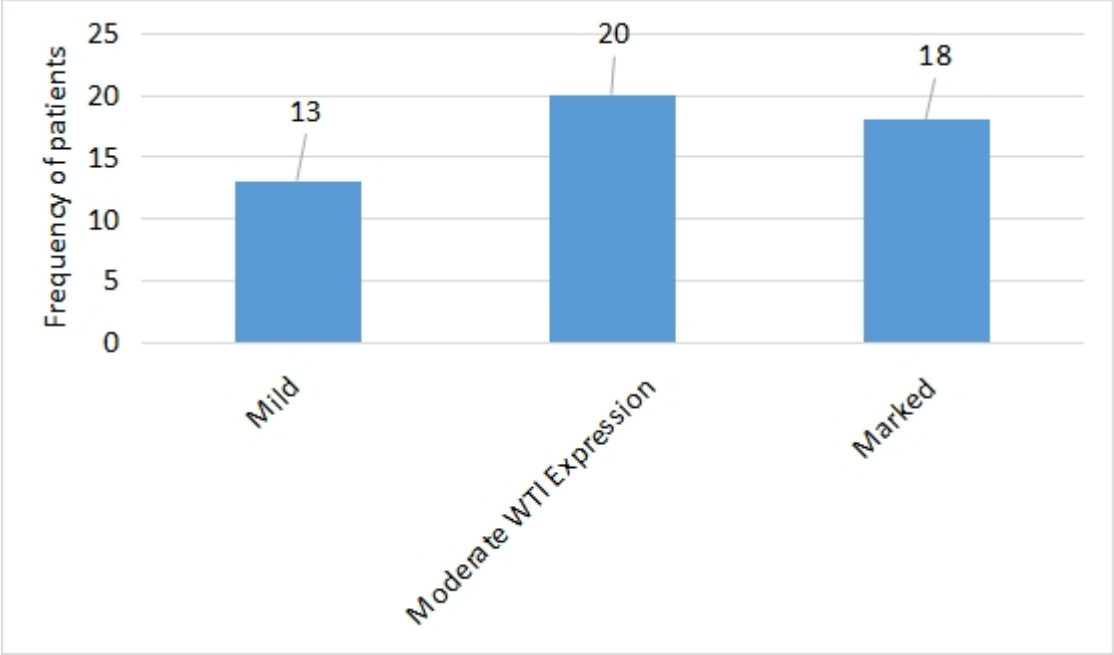


Figure 3. Scatter diagram showing positive correlation (r=0.632;p=0.001) between WT1 Expression and tumor grade

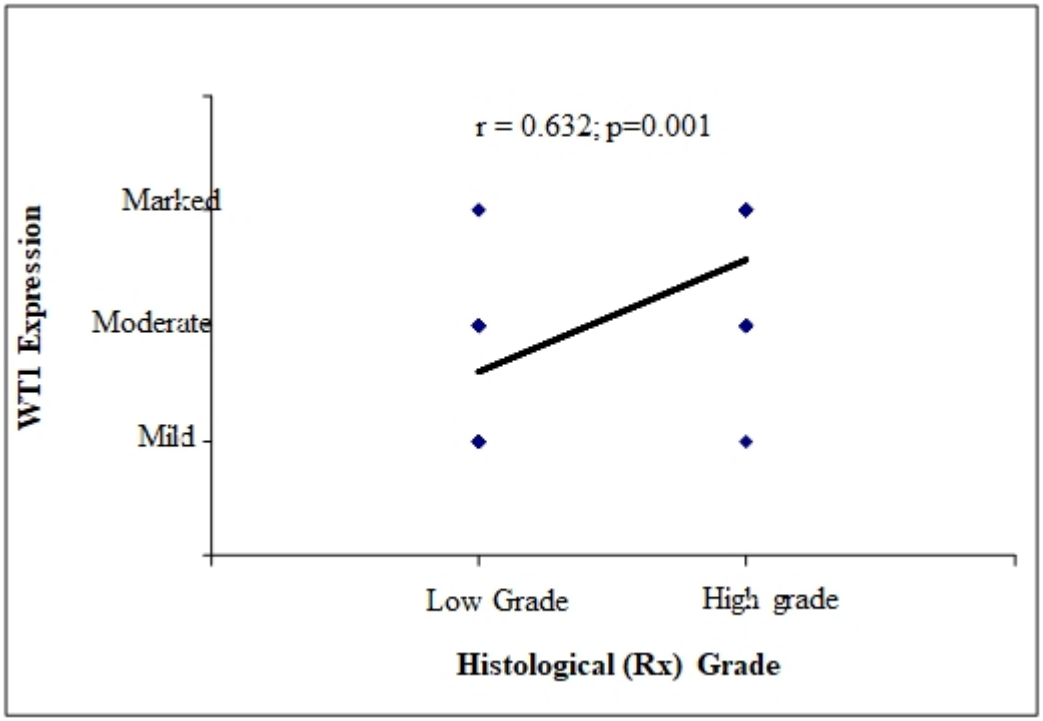
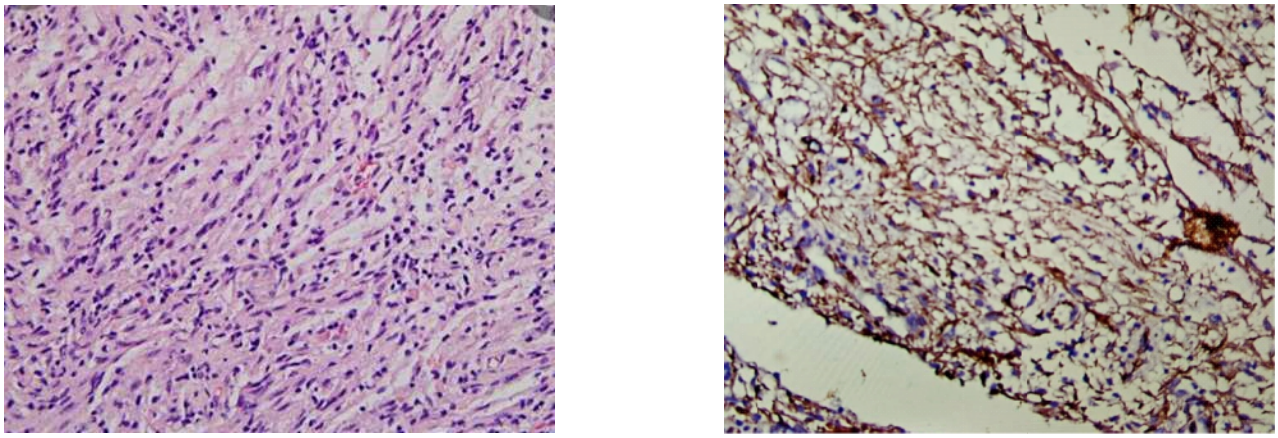
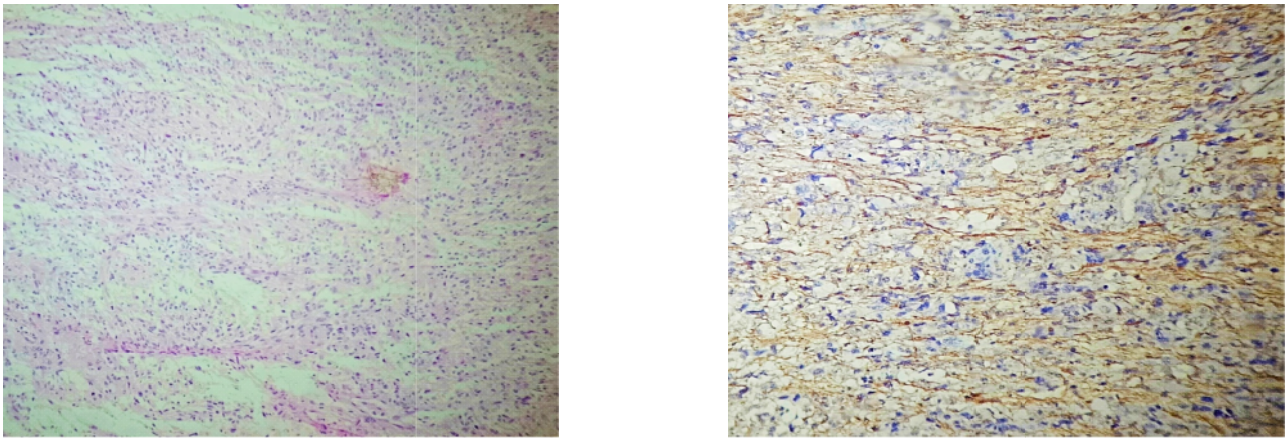


Figure 4. Bar diagram shows distribution of WT1 expression and frequency of patients.



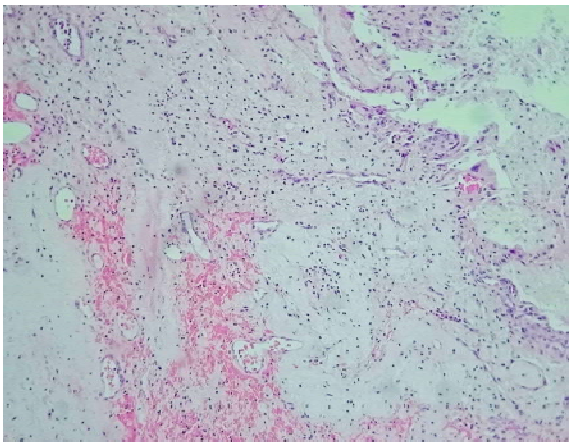
A **B**

Figure 5. (A) Photomicrograph showing a case of pilocytic astrocytoma (Grade I) H&E; 20x. (B) Photomicrograph showing marked expression of WT1 immunostain in pilocytic astrocytoma

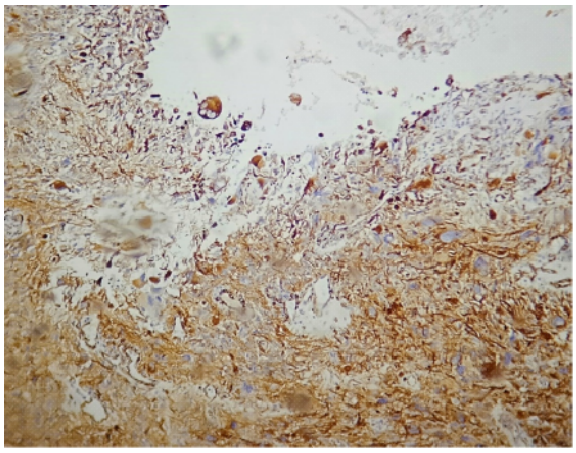


A **B**

Figure 6. (A) Photomicrograph showing a case of diffuse fibrillary astrocytoma (Grade II) H&E ;20x (B) Photomicrograph showing moderate expression of WT1 immunostain in diffuse astrocytoma



A



B

Figure 7. (A) Photomicrograph showing a case of glioblastoma multiforme (Grade IV), microvascular proliferation and pseudopalisading necrosis, H&E; 10x (B) Photomicrograph showing marked expression of WT1 immunostain in glioblastoma multiforme

Discussion

Astrocytoma's are by far the most common primary brain tumors. Although the incidence are not as high as other cancer worldwide, the prognosis and survival rates are poor because of their aggressive growth pattern and the degree of invasiveness of astrocytoma does not necessarily correlate with the grade of malignancy.[13] With the introduction of molecular targeted therapies, the outcomes for patients with a malignant glioma has improved considerably. In addition, research of these molecular markers may offer insight into the biology and pathogenesis of primary brain tumors.[14] This cross-sectional study was carried out with an aim to record WT1 expression in four grades of astrocytoma and to find its correlation with histological grade.

In the present study, the samples range from 0-70 year's age group with the highest number of sample in 3rd decade (23.4%) and the lowest from 61-70-year group. Among the 51 cases majority 30 (58.8%) were male patient and 21 (42%) were female patient. Most tumors arose in the temporal and parietal region that accounts for one fourth of the cases. This study showed slight variation from all other previous studied due to variation in sample sizes.

Out of 51 cases of astrocytoma, maximum were WHO grade IV (Glioblastoma multiforme) accounted for 18(35.3%) and WHO grade II i.e. Diffuse fibrillary astrocytoma were the second highest 15 (29.53%). WHO grade I cases, pilocytic astrocytoma accounted for 10 in number (19.6%) and the lowest were WHO grade III, namely anaplastic astrocytoma consisting 8 (15.7%). In Manocha and Jain (2019)incorporated total 79 astrocytic tumors among which most common (48.10%) were grade IV followed by grade II (34.18%). On the other hand, Ibrahim et al. (2016) the study experimented 23(25%) grade I tumor, 30 (32.60%) grade II tumor and 39(42.39%) grade IV tumor.[15-16] The variation in the number of astrocytic tumors of different grade is probably due to convenience and random availability of samples. So far in various literature GBM is the commonest considered astrocytic tumor, which even stands first in the current study (Ibrahim et al., 2016).

According to the data statistically significant results (P<0.05) was found between WHO grade and WT1 expression. It was observed that the WT1 protein in all the 51 cases of astrocytomas were expressed and there is overexpression of WT1 in high grade than low grade astrocytomas in majority of the cases. It was observed that WT1 expression was found marked in high grade astrocytomas (Grade III and IV) as many as 48 % than low grade astrocytoma (Grade- I and II) with only 1(3.4%) with marked WT1 expression. So it showed a gradual increase in WT1 expression with increasing grade. This study also found significant positive correlation between WT1 expression with histological grade in astrocytic brain tumor (Spearman's rank correlation coefficient r =0.632).

On the other hand in the current study it was observed that among the histologic parameters of prognostic value, vascular proliferation showed significant relation (p <0.05) with WT1 expression. In this study among 27(59.9%) case showed less prominent vascular proliferation, 8(15.7%) showed moderate prominent vascular proliferation and 16 (31.4%) case showed prominent vascular proliferation. In relation with WT1 immunoexpression, among the less prominent category 27 cases, 13 (65%) showed moderate WT1 expression. On the other hand, in prominent 16, more than half of the cases 10(55%) showed marked WT1 expression. Bassam et al. (2014) showed positive relationship between mean vascular density and high WT1 expression.[17] This study correlates with the above study.

With the progression of time newer methods were adapted and authors opined differently. Oji et al. (2004) and Clark et al. (2007) all found WT1 expression in many cases of astrocytoma among their study sample. However, these studies employed detection of WT1 by western blot and reverse transcriptase PCR. On the other hand, in spite of using IHC method to determine WT1 expression yielded similar results when compared WT1 molecular techniques.[18-19] Manocha and Jain (2019) although most molecular studies of WT1 have shown 100% positivity some studies using IHC approach have found absent expression in a small percentage of astrocytomas. Mahzouni et al (2012) in his study of 73 astrocytomas found WT1 protein expression in most cases (97.3%) with only two cases, one of glioblastoma and one pilocytic astrocytoma with no WT1 protein expression. Rauscher et al. (2014), in their study, found some anaplastic astrocytomas and glioblastoma lacking WT1 expression. They ascribed this negative expression in high-grade tumors to the younger age of patients and tumors possessing IDH1 mutations. In our study, none of the astrocytomas were negative for WT1, although the percentage of expression varied. So there is clear differences among authors about expression of WT1 in astrocytoma irrespective of methods used.

The current study showed that WT1 expression has positive correlation with histopathological grade.

In Bangladesh, limited studies were done in CNS tumors. According to the available information, no study was performed previously of WT1 expression in astrocytomas. So, the study results assume that WT1 expression in astrocytoma could assess tumor biology, risk of disease progression and prognosis of the tumor.

Conclusion

This study found a positive correlation of WT1 immunoexpression with WHO grades of astrocytic tumors and bear a good prognostic significance. It can, therefore, be suggested that higher expression mostly tells higher

grade of tumor and can be useful in differentiating high and low-grade astrocytomas in case of challenging biopsies**.** Routine use of WT1 in conjunction with histopathological grading in small biopsy may provide information about biological behavior of tumor, individualized risk of disease progression as well as prognosis for categorizing the patients. This categorization may aid the clinicians for further management of the patients.

References

1. Rauscher J, Beschorner R, Gierke M, Bisdas S, Braun C, Ebner F.H and Schittenhelm J. WT1 expression increases with malignancy and indicates unfavourable outcome in astrocytoma. Journal of Clinical Pathology. 2014; 67(7):556-561.
2. Rushing EJ, Sandberg GD and Horkayne-Szakaly I. High-grade astrocytomas show increased Nestin and Wilms's tumor gene (WT1) pro)(tein expression. International journal of surgical pathology. 2010; 18(4):255-259.
3. Schittenhelm J, Mittelbronn M, Nguyen T.D, Meyermann R and Beschorner R. WT1 expression distinguishes astrocytic tumor cells from normal and reactive astrocyte. Brain pathology. 2008; 18(3):344-353.
4. Ohgaki H and Kleihues P. The definition of primary and secondary glioblastoma. Clinical cancer research. 2013; 19(4): 764-772.
5. Oji Y, Suzuki T, Nakano Y, Maruno M, Nakatsuka S.I, Jomgeow T, Abeno S, Tatsumi N, Yokota A, Aoyagi S and Nakazawa T. Overexpression of the Wilms' tumor gene WT1 in primary astrocytic tumors. Cancer science. 2004; 95(10): 822-827.
6. Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, Wolinsky Y, Kruchko C and Barnholtz-Sloan J. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. Neuro-oncology. 2014; pp.iv1-iv63.
7. Rauscher J, Beschorner R, Gierke M, Bisdas S, Braun C, Ebner F.H and Schittenhelm J. WT1 expression increases with malignancy and indicates unfavourable outcome in astrocytoma. Journal of Clinical Pathology. 2014; 67(7):556-561
8. Mahzouni P and Meghdadi Z. WT1 Protein Expression in Astrocytic Tumors and Its Relationship With Cellular Proliferation Index. Advanced biomedical research. 2013; 1(3):1-5.
9. Manocha A and Jain S. WT1 in Astrocytomas: Comprehensive Evaluation of Immunohistochemical Expression and Its Potential Utility in Different Histological Grades. Indian journal of cancer. 2019; 56(3): 197-201.
10. Ohgaki H and Kleihues P. Genetic alterations and signaling pathways in the evolution of gliomas. Cancer science. 2019;100(12): 2235-2241.
11. Pala A, Karpel-Massler G, Wirtz CR and Halatsch ME. Epithelial to mesenchymal transition and progression of glioblastoma. In Clinical Management and Evolving Novel Therapeutic Strategies for Patients with Brain Tumors. 2013; 1-15.
12. Pecina-Slaus N and Kafka A. Wnt signaling and astrocytic brain tumors. CNS Oncology. 2015; 4(6): 369-370.
13. Tataranu LG, Ciubotaru V, Cazac TL, Alexandru O, Purcaru OS, Tache DE, Artene SA and Dricu A. Current Trends in Glioblastoma Treatment. In Brain Tumors-An Update 2018. IntechOpen, pp.1-15.
14. Weller M, van den Bent, M Tonn J.C, Stupp R, Preusser M, Cohen-Jonathan-Moyal E, Henriksson R, Le Rhun E, Balana C, Chinot O and Bendszus M. Evidence-based management of adult

patients with diffuse glioma–Authors' reply. The Lancet Oncology. 2017; 18(8): 66-87.
15. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW. and Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Actaneuropathologica. 2007; 114(2): 97-109.
16. Ibrahim BB, Salem MMS, Hareedy AAM and Al-Hariry N.S. Histopathological Study and Immunohistochemical Expression of WT1 Protein in Different Types and Grades of Astrocytic Tumors. 2016; 9(1): 1-6.
17. Komori T. The 2016 WHO classification of tumours of the central nervous system: the major points of revision. Neurologia medico-chirurgica. 2016: 57(7): 301-311.
18. Kros JM. Grading of gliomas: the road from eminence to evidence. Journal of Neuropathology & Experimental Neurology. 2011; 70(2):101-109.
19. Hashiba T, Izumoto S, Kagawa N, Suzuki T, Hashimoto N, Maruno M. and Yoshimine T. Expression of WT1 protein and correlation with cellular proliferation in glial tumors. Neurologia medico-chirurgica. 2017; 47(4): 165-170.