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N-Cadherin Expression in Glioblastoma and its Correlation with the Histopathological Findings

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Abstract: The aim of this study is to investigate the expression of N-cadherin in glioblastoma and to establish the relationship of this marker with the clinical and histological findings of gliblastoma. This cross sectional study was conducted on 60 cases of glioblastoma admitted to Kasr El-Eini Hospital, Faculty of Medicine, Cairo University, Egypt. We determined immunohistochemically the expression of N-cadherin in each case. N-cadherin expression was detected in 53 cases (88.3%). Among the study group, there was a significant association between expression of N-cadherin and tumor histological variant, where cases of Gliosarcoma variant showed the highest Immunoreactive score of N cadherin, while cases of glioblastoma with oligodendroglioma component showed lowest immunoreactive score of N cadherin. These findings need to be further substantiated with a prospective study supported by the use of molecular biological techniques to address the importance of N-cadherin expression as a prognostic factor for glioblastoma patients and may support the development of new therapeutics to control the tumor progression and spread among cases with strong N-cadherin expression. N-cadherin among cases of Glioblastoma is not dependent on factors like age, sex and tumor location.

Key words: Glioblastoma · N-cadherin · Immunohistochemistry

INTRODUCTION

Glioblastoma (GBM) is the most common and most lethal primary brain tumor [1]. One of the major obstacles to effective treatment and complete surgical resection of the tumor is the ability of single tumor cell for migration several centimeters away from the main tumor mass which also contributes to the high rate of tumor recurrences [2]. The interaction between cells is maintained by cell surface adhesion molecules which is considered to be a principal factor in tumor ability to spread [3]. Cadherins are a group of cell–cell adhesion molecules that are essential for cell interactions, migration and morphogenetic conversions [4]. Alterations in Cadherins function has been implicated in a number of human diseases including cancer, neuronal and mental health disorders, as well as skin and cardiovascular diseases [5]. Although their expression is not confined to these tissues but classical cadherins were originally named after the tissues within which they were first recognized [e.g. type I, epithelial (E)-cadherin and neural (N)-cadherin, type II vascular endothelial (VE)-cadherin and kidney (K)-cadherin; CDH1, CDH2, CDH5 and CDH6, respectively] [6]. Cadherin switching (epithelial-to-mesenchymal transition) is a process in which epithelial cells become motile and invasive due to loss of their polarity and cell–cell adhesions. The process is always associated with E-cadherin down regulation and N-cadherin upregulation [7]. N-cadherin expression has been identified in a number of malignant non-carcinomatous neoplasms including mesotheliomas, chordomas, synovial sarcomas, malignant melanomas, epithelioid sarcomas, epithelioid angiosarcomas and clear cell sarcomas [8] and number of carcinomatous neoplasms include renal cell carcinomas and some variant breast tumors including medullary breast carcinomas and sarcomatoid metaplastic breast carcinomas [9]. The cells of the central nervous system do not express E-cadherin but mainly N-cadherin, therefore the process of cadherin switching has not been identified in the case of malignant gliomas [10]. N-cadherin is a main regulator in the polarity of non-migrating astrocytes [11]. The invasive behavior of glioblastoma cells is partly attributed to the decreased N-cadherin expression [12]. Increasing experimental evidence suggests that N-cadherin is a potential

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therapeutic target in cancer. A peptidic N-cadherin antagonist (ADH-1) has been developed and has entered clinical testing [13]. The aim of the study is to investigate the expression of N cadherin in glioblastoma and to establish the relationship of this marker with the clinical and histological findings of gliblastoma.

MATERIALS AND METHODS

The material of this retrospective cross-sectional study consisted of 60 cases diagnosed as glioblastoma. The paraffin blocks were received from the Pathology department, Faculty of medicine, Cairo University, Kasr El Eini Hospital over a two year period from January 2011 to December 2012. The personal data (age and sex), clinical details and pathological data pertaining to these patients were retrieved from the medical records department of Kasr El Eini Medical College Hospital. Two sections were cut from each paraffin block by microtome at 5 microns thickness; One stained with hematoxylin and Eosin for routine histopathoogical examination, The other was mounted on positively charged slides for immunohistochemical staining of proliferating astrocytic cells by N-Cadherin.

Immunohistochemical Staining for N-Cadherin: The Sections were deparaffinized and transferred into three changes of xylene, then slides were rehydrated through decreasing concentrations of alcohol. The slides were washed in phosphate buffer saline PBS for 5 minutes. The endogenous peroxidase activity was blocked using a 3 % solution of hydrogen peroxide for 20 minutes at room temperature then slides were washed well in water. Sections were placed in an unsealed thermorsistent plastic jar filled with 10 ml of citrate buffer (pH 6) then microwaved on high power for three times 5 minutes each. The jar was removed and allowed to cool for 20-30 minutes, then slides were washed well with PBS for 5 minutes. Tissue sections were covered immediately with 2 drops of blocking agent (normal rabbit serum) on each slide and were left for 30 minutes. Each slide was incubated with one or two drops of 1/150 diluted N-cadherin mouse monoclonal antibody (N Cadherin Antibody (13A9), Code No. NBP1-48309, DAKO) in primary antibody diluents (Genemed). Slides were incubated horizontally in a humid room temperature for 120 minutes. Sections were washed three times in PBS and incubated with avidin-biotin-peroxidase system for 30 minutes. Brown color was developed using diaminobenzidine (DAB) which was applied for 4-5 minutes then slides were washed in buffer. Antigen binding sites were visualized then counterstaining with hematoxyline was done. Slides were washed in tape water then dehydrated in ascending grades of alcohol. Slides were then cleared in 2 changes of xylene. Slides were left to dry in air then a drop of Canada balsam was added and sections were covered by a glass cover. Controls: As negative controls, primary antibody was replaced by PBS and processed as above. As positive controls, immunoreactivity of normal neuropil embedded in neoplastic sections was evaluated.

Evaluation of N-Cadherin Immunostaining: N-Cadherin marker expressed at the cell membrane or within the cytoplasm, the immunoreactive score (IRS) was applied for evaluating both the intensity of immunohistochemical staining and proportion of stained cells. The immunoreactive score (IRS) gives a range of 0–12 as a product of multiplication between positive cells proportion score (0–4) and staining intensity score (0–3). Positive cells were quantified as a percentage of the total number of cells and assigned to one of five categories: 0, no positive cells; 1, <10; 2, 10–50; 3, 51–80; 4, >80%. The staining intensity was subclassified as 0 (No stain), 1 (weak), 2 (moderate) and 3 (strong). The percentage of positivity of the tumor cells and staining intensity were multiplied to generate the immunoreactive score for each tumor specimen (Table 1). For a diagnostic purpose, a cut-off of 10% stained tumor cells was chosen to define a positive tumor [14].

Statistical Analysis: Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21. Comparisons between two groups with respect to normally distributed numeric variables were done using the t-test. Non-normally distributed numeric variables were compared by Mann-Whitney test. For categorical variables, differences were analyzed with 2 (Chi square) tests and Fisher's exact test when appropriate. All p-values are two-sided. P-values < 0.05 were considered significant.

RESULTS

This retrospective study was conducted on sixty cases diagnosed as glioblastoma over a two year period from January 2011 to December 2012 in Kasr El Eini Hospital, Faculty of Medicine, Cairo University, Egypt. As regards the histological variants of glioblastoma among the studied cases, 49 (82%) cases were classified

Table 1: The immunoreactive score (IRS) [14]

A (percentage of positive cells)	B (intensity of staining)	IRS score (multiplication of A and B)
0 = no positive cells	0 = no color reaction	0-1 = negative
1 = <10% of positive cells	1 = mild reaction	2-3 = mild
2 = 10-50% positive cells	2 = moderate reaction	4-8 = moderate
3 = 51-80% positive cells	3 = intense reaction	9-12 = strongly positive
4 = >80% positive cells	Final IRS score (A × B): 0-12	--

Table 2: Relationship between N-cadherin and clinicopathological variables

	N cadherin expression					
Factors	Negative (n=7)	Mildly positive (n= 8)	Moderately positive (n= 26)	Strongly positive (n= 19)	P value	Significance
Age (years)						
Mean ±SD	51.4±15.05	46.75±9.3	49.04±16.8	48.16±15.06	0.876	Insignificant
Sex						
Male	5	6	18	13	0.848	Insignificant
Female	2	2	8	6		
Tumor location						
Occipital	0	0	2	1		
Parietal	3	1	6	7		
Frontal	1	4	7	3		
Temporal	3	1	2	5		
Temporo-parietal	0	1	4	1	0.559	Insignificant
Fronto-parietal	0	0	2	0		
Occipito-parietal	0	0	1	0		
Cerebellum	0	0	0	1		
Others	0	1	2	1		
Histological variant						
Classical	6	5	23	15		
GS	0	0	2	3	0.012	Significant
GBMO	1	3	0	0		
Pediatric HGG	0	0	1	1		

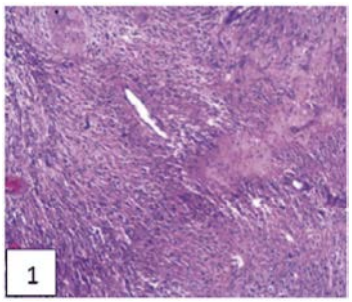

Fig. 1: Glioblastoma, classic variant (H&E, 100x)

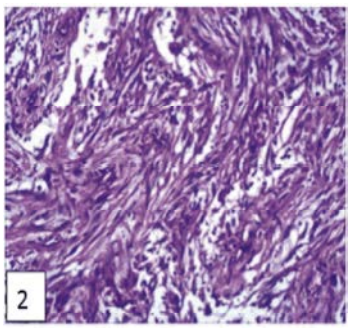

Fig. 2: Gliosarcoma (H&E, 200x)

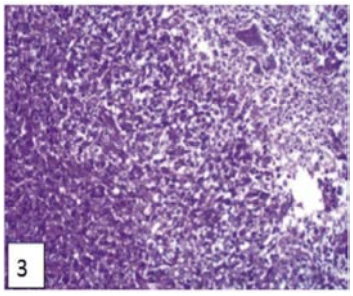

Fig. 3: Glioblastoma with oligodendroglioma component (H&E, 200x)

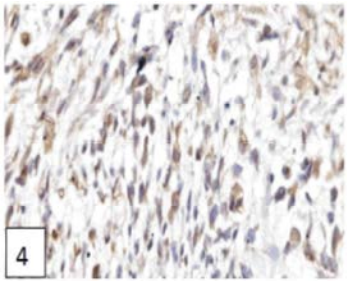

Fig. 4: N-cadherin mildly positive (200x)

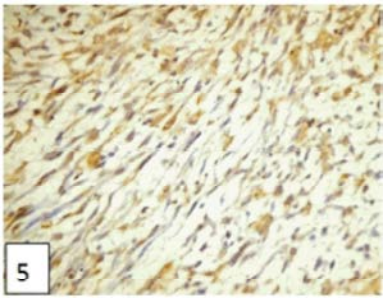

Fig. 5: N-cadherin moderately positive (100x)

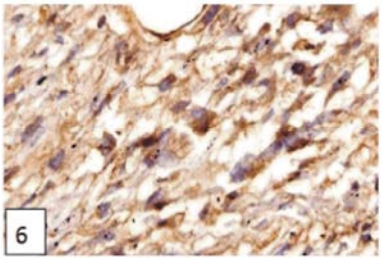

Fig. 6: N-cadherin strongly positive (400x)

as classical variant of glioblastoma (Fig. 1), 5 (8%) cases were classified as gliosarcoma (Fig. 2), 4 (7%) cases were classified as glioblastoma with oligodendroglioma component (Fig. 3) and 2 (3%) cases as pediatric high grade glioma. Among the classical variant 34 (69.4%) cases were denovo cases and 15 (30.6%) cases had history of previous excision with local recurrence; 12 (24.5%) cases were previously diagnosed as glioblastoma (primary GBM) while 3 (6.1%) cases were previously diagnosed as astrocytomas with lower grade of malignancy (Secondary GBM). The age of patients in this study ranged from 6 year to 82 years with mean age 48.73 ±11.75 years. Median age was 50.5 years. Statistical analysis showed that the two peak incidences of GBM were found in the fifth and sixth decade of life. Male preponderance (42 male and 18 female patients) was noted with a female to male ratio of 1:2.33. As regards the location of the lesions, the parietal lobe was the commonest site involved. It was involved alone in 17 cases representing 28.3% of the cases and it was involved together with other brain lobes in 9 cases representing 15 % of cases with total parietal lobe involvement in 26 cases representing 43.3% of cases. The cerebral hemispheres were involved in 55 (91.7%) cases; 46 of which involved single lobe (76.67%) and 9 (15%) of which involved more than one lobe. 4 (6.7%) cases involved deeper brain structures and one (1.6%) case involved the cerebellum. As regards expression of N-cadherin, it was present within the cytoplasm and at cell borders in 53 (88.3%) cases; 8 (13.3%) cases were mildly positive (Fig. 4), 26 (43.3%) cases were moderately positive (Fig. 5), 19 (31.7%) cases were strongly positive (Fig. 6) and 7 (11.7%) cases were considered negative for N cadherin. The mean IRS of N cadherin among studied cases was 5.67±3.51. The median value was 5.

Correlation between the clinico-pathological variables and expression of N-cadherin among the study group showed none statistically significant correlation except for the tumor histological variant and N-cadherin expression (Table 2). Expression was notably significant among gliosarcoma cases with mean IRS value = 9±3, while cases of glioblastoma with oligodendroglioma component showed lowest IRS of N cadherin with mean value = 1.75±0.5. Expression of N-cadherin among recurrent cases (Mean IRS: 5.8) was slightly higher than denovo cases (Mean IRS: 5.44) of classical variant of GBM, however correlation between IRS of N cadherin among studied cases with classical variant of GBM showed no significant correlation with the tumor recurrence (P value = 0.065).

DISCUSSION

Several studies were carried out in order to characterize N-cadherin expression and function in GBMs. Shinora *et al.* [15] and Asano *et al.* [16] stated that N-cadherin expression is directly correlated with the glioma grade. In addition, Utsuki *et al.* [17] demonstrated that N-cadherin protein levels are increased in GBMs where they investigated the relationship between E-cadherin, N-cadherin and β-catenin expression and tumor grade in astrocytomas. In their study, N-cadherin was present at cell-cell borders in 61% of 18 glioblastoma cases which is much less than our results. In contrast to Reszec *et al.* [18] on their study on 61 cases of glioblastoma where N-cadherin expression was observed in all cases. Kouhotek *et al.* [19] stated that N-cadherin is posttranslationally cleaved at higher levels in glioblastoma cells than in normal astrocytes and that cleaved N-cadherin is present in human GBM specimens and this finding may help to explain the seemingly conflicting data regarding the expression of N-cadherin in glioblastomas and that modification such as post translational cleavage, which occurs more abundantly in neoplastic astrocytes, may confound studies examining overall protein expression. Therefore, examining the function of N-cadherin, rather than merely its expression, may be more informative in elucidating its role in gliomagenesis. Asano *et al.* [16] initially showed that at the time of glioma recurrence decreased N-cadherin

expression correlated with tumor invasion. In contrast to our study, N-cadherin IRS among recurrent cases was slightly higher than that of Denovo cases; however no statistical significant correlation was found. In Wu *et al.* [20], no significant differences in the expression levels of â-catenin and N-cadherin were observed for age, sex, location or diffuse growing pattern. The same results concerning N cadherin were seen in the presenting study.

CONCLUSION

There is an association between N cadherin expression and the histological variants of glioblastoma, where Gliosarcomas express strong N cadherin immunoreactivity while Glioblastomas with oligodendroglioma component express weak N cadherin immunoreactivity, with a direct correlation between N cadherin expression and the Glioblastoma variants. N-cadherin may need to be re-evaluated as possible biomarkers or therapeutic targets in the treatment of gliosarcoma. Researchers and pathologists therefore should clearly delineate between different variants of Glioblastoma due to the divergent expression patterns in immunohistochemistry among different variants of GBM and within the same tumor tissue. Our study emphasizes that N cadherin among cases of Glioblastoma is not dependent on factors like age, sex and tumor location. Further studies are needed to clarify the role of N cadherin in Glioblastoma rate of recurrence and patient survival.

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