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RESEARCH ARTICLE

PODOPLANIN AND KI-67 AS FUTURE PROGNOSTIC MARKERS IN GLIOMAS

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ABSTRACT

Background and Objective: Gliomas are the most common central nervous system tumors. Prognostic and predictive markers play an important role in clinical practice for the assessment of prognosis and the selection of appropriate therapy. The aim of this study to assess the immunohistochemistry status of podoplanin and Ki67 in low grade and high grade gliomas and correlating them with histopathological grading, treatment and overall survival of the patients. ***Material and Methods:*** This analytical study of the 2 year duration on 150 glial tumor tissue was done which were processed accordingly and grading was done after H &E staining. The scoring of podo was divided into podo score 1: focal staining (0-2) and podo score 2: extensive staining (3-4).The scoring of Ki67 was calculated as Score 1 :< 4%, Score 2 :4-10 & Score 3 :> 10%. ***Result:*** Majority of cases having podo score 1 were of low grade glioma while majority of cases with podo score 2 were high grade glioma. Podoplanin and Ki-67 scoring increased with increasing grade of glial tumors. Mean survival duration of cases who received radiotherapy was significantly higher as compared to those who did not receive radiotherapy. ***Conclusion:*** Till present date the long term survival of patients suffering from malignant gliomas remains low, which points the need for new independent prognostic factors. In search of this emerging need we found of podoplanin and Ki-67 really promising.

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INTRODUCTION

Gliomas include the most aggressive forms of adult primary brain tumours, they are characterized by high degrees of intratumoral heterogeneity that complicate their treatment[1].Gliomas are either astrocytic, oligodendrocytic, ependymal or a mix of these 2 cell types[2]. Despite recent advances in neuro-imaging, neurosurgical resection techniques and the development of novel adjuvant therapies, the long-term survival of patients suffering from malignant glioma remains low[3]. Podoplanin has been suggested to be important for spheroid formation thereby suggesting a role in stemness in glioma [4]. In addition, Podoplanin is involved in cancer cell migration, invasion, metastasis and malignant progression [5-6], although it affects the activities of RhoA and ERM (Ezrin, Radixin, Moisin) proteins, which link cell membranes and the cytoskeleton. The common use of PDPN is identification of lymphatic invasion and the detection of mesothelioma, meningioma, squamous cell carcinoma of oral cavity and female genital tract malignancy, Ki-67 is a nuclear protein that may be necessary for cellular proliferation. Ki-67 can be used as a marker to assess the growth portion of a given cell population, as this protein is present in all proliferating cells[7].

The monoclonal antibody Ki-67/MIB-1 detects this antigen and the percentage of immunopositive cells is referred to as the Ki-67 labeling index (LI)[8]. The monoclonal antibody Ki-67/MIB-1 has proven prognostic and diagnostic power in astrocytic tumors[9]. The study was conducted to evaluate the immunohistochemistry status of podoplanin and Ki67 in low grade and high grade gliomas and correlating them with histopathological grading and survival of the patients.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology, King George's Medical University, U.P., Lucknow from from Sep 2016 to Sep 2018. Glioma specimens were taken on the basis of clinicoradiological findings with adequate patient's clinical information and proper consent. Specimen was processed according to standard protocols and grading was done after staining with Hematoxylin and Eosin stain (H&E). Gliomas were classified according World Health Organization guidelines and were divided into grade I, II, III and IV. The cases of Grade I and Grade II tumours were categorized in the low grade gliomas while Grade III and Grade IV were categorized in high grade gliomas.

Immunohistochemistry was performed with antibodies to podoplanin and Ki-67. Podoplanin was manufactured by Abcam(Rabbit monoclonal to podoplanin diluted in PBS, ph 7.6, in a dilution of 1:250) and Anti Ki-67 was manufactured by Biogenex (Pre-diluted ready–to-use Mouse monoclonal antibody to Ki-67 antigen)

Scoring of IHC-All slides were examined by light microscopy.

For the interpretation of PDPN IHC: Membranous staining of the tumor cells was used. The positivity was assessed in two parts:

Intensity of the staining: The intensity was scored as -No detectable Podoplanin immunostaining (score 0),Focal membranous expression levels (score 1+),Extensive membranous expression level (score 2+). **(2)Percentage of cells stained:** It was expressed as a percentage of the total number of cancer cells examined (1000 cells per specimen), No positive tumor cell (score 0) ,10-30% positive tumor cells(score 1+),>30% positive tumor cells (score 2+). To assess the final scoring of podoplanin, intensity of staining scoring is added with scoring of% of cells stained. The value ranges from 0-4 and is divided into two grades, Score 1(focal membranous staining): 0-2,Score 2 (extensive membranous staining):3-4.

For the interpretation of Ki67 IHC: It was calculated as the percentage of positively stained tumor cell nuclei out of the total tumor cells counted (n=1000) and the scoring was done as follows: ≤4% (Score 1),5-10% (score 2),≥10% (score3).

Statistical analysis: The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and mean±SD. The statistical evaluation was carried out using the generalized Wilcoxon test. The p values and significance of data were checked by Chi-square test.

RESULTS

This study evaluate expression of Podoplanin and Ki-67 in Low grade and High grade Gliomas and correlate immunohistochemistry status of the above two with the histomorphological grading,treatment and overall survival rate. Total 150 cases were studied which included 62 cases of low grade glioma (26 Pilocytic Astrocytoma, 16 Diffuse Astrocytoma, 8 Ependymoma, 4 Oligodendroglioma, 2 Diffuse Fibrillary Astrocytoma, 1 Gemistocytic Astrocytoma, 2 Myxopapillary Ependymoma, 3 subependymal giant cell Astrocytoma) and 88 cases of high grade glioma (61 Glioblastoma, 11 Anaplastic Astrocytoma, 6 Gliosarcoma, 5 Anaplastic Ependymoma, 3 Anaplastic Oligodendroglioma, 1 Anaplastic oligoastrocytoma and 1 pilocytic Astrocytoma with anaplastic features) along with 5 normal brain specimen as control. Among low grade glioma cases most common histological diagnosis was pilocytic astrocytoma (41.9%) and high grade glioma cases was Glioblastoma (69.3%)(Table-1).In overall 62 low grade glioma cases the population with highest number of cases 26(69.2%) is ≤20yrs.In overall 88 high grade glioma cases the population with highest number of cases 33(54.1%) is 41-60yrs. Difference in age of high grade glioma cases with different histological diagnosis was found to be statistically highly significant(Table-2).Duration of symptoms among patients of glioma ranged between 0.2-68 months (median 2 months). Mean duration of symptoms of study population was 5.59±8.95. Mean duration of symptoms among cases of Low grade glioma (7.76±8.90 m) was found to be higher as compared to High grade glioma (4.06±8.71 m) cases. This difference was found to be statistically significant (Table-3). Majority of the cases of glioma were High grade glioma at Frontal (68.4%), Frontotemporal (100.0%), Temporoparietal (83.9%), Parieto-occipital (94.4%) while majority of the cases were low grade glioma at Posterior fossa (83.3%), Spine (100.0%) and Other sites (66.7%). This difference was found to be statistically significant (Table-4). Majority of the patients with Podo score 1 had Low grade glioma (72.4%) while majority of the patients with Podo score 2 had High grade glioma.

Association of Podo score and Grade of glioma was found to be statistically significant(Table-5).Majority of patients with Ki67 score 1 had low grade glioma (74.7%) while majority of cases were High grade glioma having Ki67 score 2 (83.3%) and Ki67 score 3(100%). Association of Ki67 score and Grade of glioma was found to be statistically significant (Table-6).Majority of cases with podo score 1 having Ki-67 score 1 (75.9%) while majority of cases with Podo score 2 having Ki67 score 2 (66.7%) and Ki67 score 3 (81.1%). Association of Ki67 score and Podo score was found to be statistically significant (p<0.001)(Table-7).

Survival: Out of 150 patients enrolled in the study 22 (14.6%) lost to follow up, 11 patients expired to post-op complication, 57 (38.0%) patients survived and rest 60 (40%) expired due to glioma. The patients who were lost to follow up and patients who died due to post-op complication were excluded from the survival studies and data of 117 patients is included in the survival studies done for the period of 21 months.Out of 117 patients who could be followed up, survival rate was 38.0% (57/117) while rate of expiry was 40% (60/117) (Table-8). Proportion of expiry was higher among high grade glioma as compared to low grade glioma. Out of 117, 49 cases of Low grade glioma who could be followed, 10 (20.4%) expired while among 68 cases of High grade glioma, 50 (73.5%) expired. Overall survival time 12.61±1.03was months among study population. Mean survival duration of Low grade glioma patients (19.57±1.25 months) was significantly higher as compared High grade glioma cases (7.28±1.13 months). This difference was found to be statistically highly significant (p<0.001) (Table-9).

Mean survival of patients with podo score 1(18.34±1.26) was significantly higher as compared to patients with podo score 2 (6.74±1.17).This difference was found to be statistically significant)(Table-10).Mean survival of patients with Ki-67 score 1(17.58±1.27months) was significantly higher as compared to patients with Ki67 score 2 (10.48±2.27months)& 3 (5.19±1.23months). This difference was found to be statistically significant (p<0.001)(Table-11).

Survival Analysis for Glioma cases with Status of Radiotherapy: Out of 117, 22 patients of glioma who received radiotherapy, only 1 (4.5%) expired while among 95 cases who did not receive radiotherapy, 59 (62.1%) expired. Mean survival duration of cases who received Radiotherapy (22.00±0.98 months) was significantly higher as compared to those who did not receive Radiotherapy (10.31±1.10months).Out of 95 cases who did not receive radiotherapy, 10 of 40 Low grade glioma (25%) expired while 49 of 55 High grade glioma expired (90.5%). Mean survival duration of low grade glioma(18.63±1.48 months) higher as compared to high grade glioma(3.60±0.65 months) (Table-12).

DISCUSSION

Gliomas are the most common form of brain tumors, contributing to more than half of the incidence of brain tumors. PDPN is detected in glioma stem cells, which interact extensively with the tumor microenviorment[1]. It also promotes tumour invasion through re-organization of cytoskeleton of neoplastic cells not only by combined cell migration, but also by individual cell migration after loss of E-Cadherin and thus play role in tumourogenesis[10]. The present analytical study correlates the expression of Podoplanin with the increasing grade of glioma hence providing information about its use as a future prognostic marker, also the inhibitors of podoplanin could be used as a potent strategy for treating cancer as it's upregulation is seen in malignancies. The study was included 150 cases which were graded histomorphologically according to WHO Classification of Tumors of the Central Nervous System into Grade I to Grade IV. The cases of Grade I and Grade II tumors were categorized in low grade gliomas and the cases with Grade III and Grade IV were categorized in high grade gliomas. On categorization we included 62 cases of Low grade Gliomas, 88 cases of high grade gliomas and 5 control cases (Autopsy Brain tissue).

Table 1. Distribution of cases according to histological diagnosis of tumours

Low grade glioma	No. of cases	% of low grade glioma (n=62)	Percentage overall (n=150)
Pilocytic Astrocytoma	26	41.9	17.3
Diffuse astrocytoma	16	25.8	10.7
Ependymoma	8	12.9	5.3
Oligodendroglioma	4	6.5	2.7
Subependymal giant cell astrocytoma	3	4.8	2.0
Diffuse fibrillary astrocytoma	2	3.2	1.3
Myxopapillary Ependymoma	2	3.2	1.3
Gemistocytic Astrocytoma	1	1.6	0.7
Total	62		41.3

High grade glioma	No. of cases	% of high grade glioma (n=88)	Percentage overall (n=150)
Glioblastoma	61	69.3	40.7
Anaplastic Astrocytoma	11	12.5	7.3
Gliosarcoma	6	6.8	4.0
Anaplastic Ependymoma	5	5.7	3.3
Anaplastic oligodendroglioma	3	3.4	2.0
Anaplastic oligoastrocytoma	1	1.1	0.7
Pilocytic astrocytoma with anaplastic features	1	1.1	0.7
Total	88		58.7

Table 2. Association of Age and Histological Diagnosis (Low Grade Glioma) (n=62)

Diagnosis	Total	≤20 yrs		21-40 yrs		41-60 yrs	
		No.	%	No.	%	No.	%
Diffuse Astrocytoma	16	7	43.8	7	43.8	2	12.5
Diffuse fibrillary astrocytoma	2	1	50.0	0	0.0	1	50.0
Ependymoma	8	7	87.5	1	12.5	0	0.0
Gemistocytic Astrocytoma	1	0	0.0	1	100.0	0	0.0
Myxopapillary ependymoma	2	1	50.0	1	50.0	0	0.0
Oligodendroglioma	4	0	0.0	2	50.0	2	50.0
Pilocytic Astrocytoma	26	18	69.2	8	30.8	0	0.0
Subependymal giant cell astrocytoma	3	2	66.7	1	33.3	0	0.0
Total	62	36	58.1	21	33.9	5	8.1

χ^2=26.046(df=14); p=0.026 (Sig) ; Min-Max (Median): 3-55 (19.00); 21.29±12.76 years

Association of Age and Histological Diagnosis (High Grade Glioma) (n=88)

Diagnosis	Total	≤20 yrs		21-40 yrs		41-60 yrs		>60 yrs	
		No.	%	No.	%	No.	%	No.	%
Anaplastic astrocytoma	11	1	9.1	7	63.6	3	27.3	0	0.0
Anaplastic ependymoma	5	4	80.0	1	20.0	0	0.0	0	0.0
Anaplastic oilgodendroglioma	3	0	0.0	1	33.3	1	33.3	1	33.3
Anaplastic Oligoastrocytoma	1	0	0.0	1	100.0	0	0.0	0	0.0
Glioblastoma	61	4	6.6	16	26.2	33	54.1	8	13.1
Gliosarcoma	6	1	16.7	1	16.7	4	66.7	0	0.0
Pilocytic Astrocytoma with anaplastic features	1	1	100.0	0	0.0	0	0.0	0	0.0
Total	88	11	12.5	27	30.7	41	46.6	9	10.2

χ^2=43.527(df=18); p=0.001 (Sig) ; Min-Max (Median): 9-72 (45.00); 41.94±16.01 years

Table 3. Comparison between Duration of Symptoms with grade of tumor

Duration of symptoms	Overall (n=150)		Low Grade Glioma (n=62)		High Grade Glioma(n=88)	
	No.	%	No.	%	No.	%
<3 m	80	53.3	20	32.3	60	68.2
3-6 m	45	30.0	24	38.7	21	23.9
6-12 m	9	6.0	8	12.9	1	1.1
>12 m	16	10.7	10	16.1	6	6.8
			χ^2=22.823(df=3); p<0.001 (Sig)			
Min-Max (Median)	0.20-68 (2) m		0.84-36 (4.5) m		0.20-68 (2) m	
Mean±SD	5.59±8.95		7.76±8.90		4.06±8.71	

Table 4. Association of Low and High Grade Glioma with Site of Tumour

Site of Tumour	Overall (n=150)		Low Grade Glioma (n=62)		High Grade Glioma(n=88)	
	No.	%	No.	%	No.	%
Frontal	38	25.3	12	31.6	26	68.4
Frontotemporal	5	3.3	0	0.0	5	100.0
Temporoparietal	31	20.7	5	16.1	26	83.9
Parieto-occipital	18	12.0	1	5.6	17	94.4
Posterior fossa	24	16.0	20	83.3	4	16.7
Spine	4	2.7	4	100.0	0	0.0
Other	30	20.0	20	66.7	10	33.3
			χ^2=53.713(df=6); p<0.001 (Sig)			

Table 5. Association of Podo Score and Grade Glioma

Podo Score	Overall (n=150)		Low Grade Glioma (n=62)		High Grade Glioma(n=88)	
	No.	%	No.	%	No.	%
Podo score 1(0-2)	76	50.7	55	72.4	21	27.6
Podo score 2(3-4)	74	49.3	7	9.6	67	90.4
			χ^2=61.191(df=1); p<0.001 (Sig)			

Table 6. Association of Ki67 Score and Grade Glioma

Ki67 Score	Overall (n=150)		Low Grade Glioma (n=62)		High Grade Glioma(n=88)	
	No.	%	No.	%	No.	%
Ki67 score 1	79	52.7	59	74.7	20	25.3
Ki67 score 2	18	12.0	3	16.7	15	83.3
Ki67 score 3	53	35.3	0	0.0	53	100.0
			χ^2=77.233(df=2); p<0.001 (Sig)			

Table 7. Association of Ki67 Score and Podo score

Ki67 Score	Overall (n=150)		Podo score 1 (n=76)		Podo score 2 (n=74)	
	No.	%	No.	%	No.	%
Ki67 score 1	79	52.7	60	75.9	19	24.1
Ki67 score 2	18	12.0	6	33.3	12	66.7
Ki67 score 3	53	35.3	10	18.9	43	81.1
			χ^2=43.807(df=2); p<0.001 (Sig)			

Table 8: Distribution according to Survival

Outcome	No. of cases	Percentage
Survived	57	38
Expired	60	40
Expired due to post-op complications	11	7.3
Lost to Follow-up	22	14.6
Total	150	100.00

Table 9: Survival Analysis for Low and High Grade of Glioma

Grade of Glioma	Total N	No. of Mortalities	%	Mean survival time±SE
Low	49	10	20.4	19.57±1.25
High	68	50	73.5	7.28±1.13
Overall	117	60	51.3	12.61±1.03

Log Rank (Mantel Cox) χ2=32.862; p<0.001

Table 10. Survival Analysis for Glioma cases with different Podo scores

Podo score	Total N	No. of Mortalities	%	Mean survival time±SE
Podo score 1(0-2)	58	15	25.9	18.34±1.26
Podo score 2(3-4)	59	45	76.3	6.74±1.17
Overall	117	60	51.3	12.61±1.03

Log Rank (Mantel Cox) χ2=33.575; p<0.001

Table 11. Survival Analysis for Glioma cases with different Ki67- scores

Ki67 score	Total N	No. of Mortalities	%	Mean survival time±SE
Ki67 score 1	61	18	29.5	17.58±1.27
Ki67 score 2	14	7	50.0	10.48±2.27
Ki67 score 3	42	35	83.3	5.19±1.23
Overall	117	60	51.3	12.61±1.03

Log Rank (Mantel Cox) χ2=38.422; p<0.001

Table 12. Survival Analysis for Glioma cases with Status of Radiotherapy

Radiotherapy	Total N	No. of Mortalities	%	Mean survival time ±SE
Radiotherapy received	22	1	4.5	22.00±0.98
Radiotherapy not received	95	59	62.1	10.31±1.10
Overall	117	60	51.3	12.61±1.03

Log Rank (Mantel Cox) χ2=18.193; p<0.001

Association of Podoplanin with Grade of glioma: In our study majority cases having podoplanin score 2 (3-4) were High grade glioma (90.4%) (Figure 1) while majority of cases having podo score 1 (0-2) were Low grade glioma (72.4%)(Figure 2,3). Association of podo score and grade of glioma was found to be statistically significant (p<0.001).

In normal brain tissue specimens taken as control as well as the tumor specimens with areas of normal brain tissue, showed weak podoplanin. Podoplanin staining was also observed around the areas of endothelial cell proliferation and necrotic areas depicting its role in the process of cancer cell migration, invasion, metastasis, malignant progression and platelet aggregation (Figure 4-7).

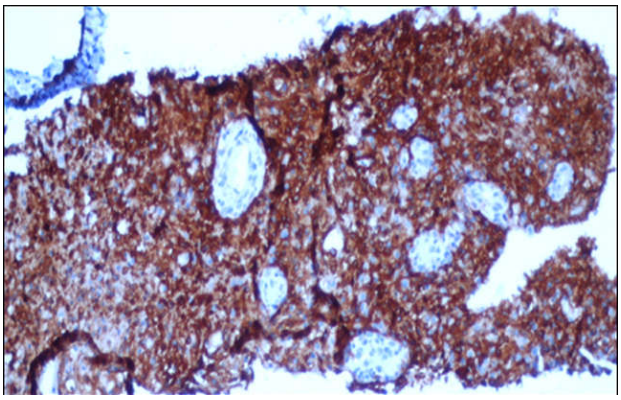

Figure 1: Podoplanin strong expression 2+(>30%) in glial tumour cells

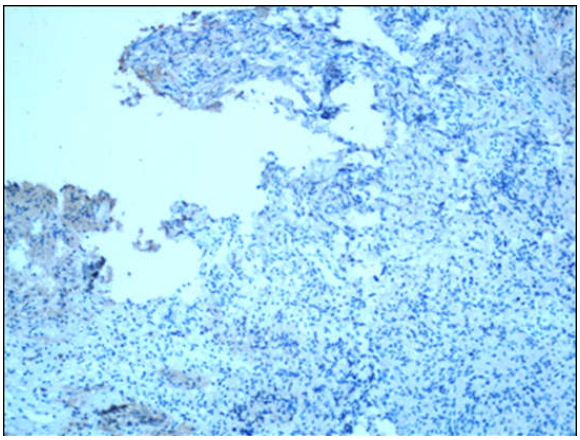

Figure 2. Podoplanin low or absent expression 0 (0-10%) in glial tumor cells

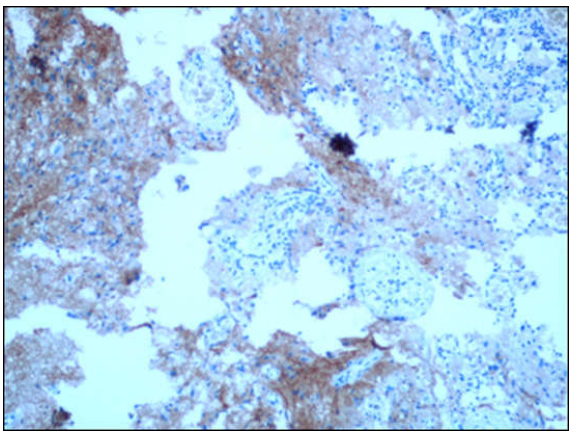

Figure - 3: Podoplanin focal expression 1+(10-30%) in glial tumour cells

In our study we found that staining intensity ranged from 0-10%, 10-30%, and >30%. These observations were in concordance with the study done by Mishima K et al. (2006)[11] who studied 188 brain tissue specimens including 30 diffuse astrocytoma, 43 anaplastic astrocytoma and 115 glioblastoma. Popoplanin was expressed on the surface of anaplastic astrocytoma and glioblastoma cells, especially around necrotic areas and proliferating endothelial cells. On the other hand, podoplanin expression was not observed in diffuse astrocytoma. Similar results was shown in the study done by Stefen J Grau et al. (2015)[12] who studied 40 cases of gliomas including 20 glioblastoma(GBM) and 20 low grade astrocytomas. They found that all GBM specimens showed a strong PDPN expression, which were mainly focused on areas of high vessel density with a strong signal on tumour cells adjacent to vessels. Endothelium itself did not show reactivity.

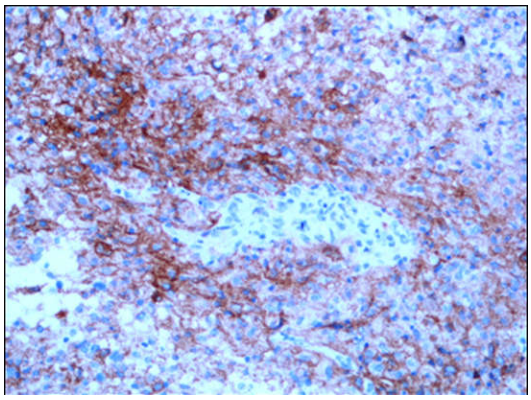

Figure 4. Podoplanin strong (2+) expression around cells near the vessel wall

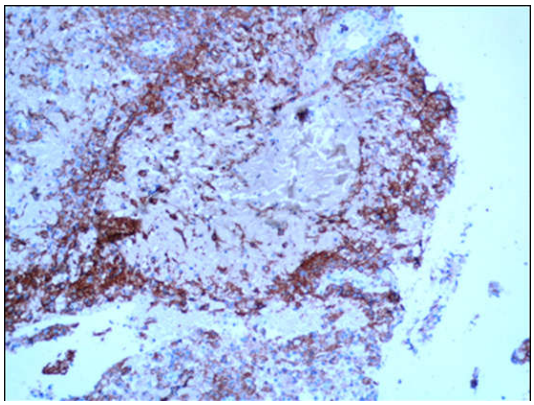

Figure - 5: Podoplanin strong (2+) expression around cells near the necrosis

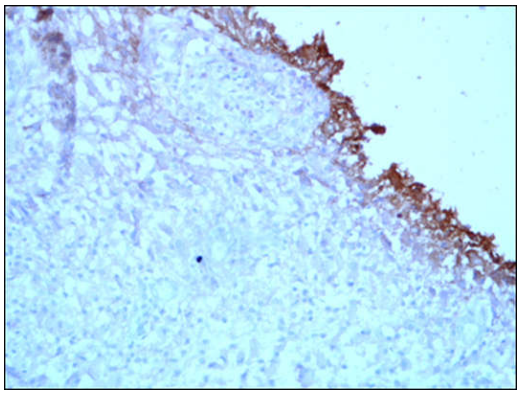

Figure 6. Podoplanin strong (2+) expression at periphery of tumour cell.

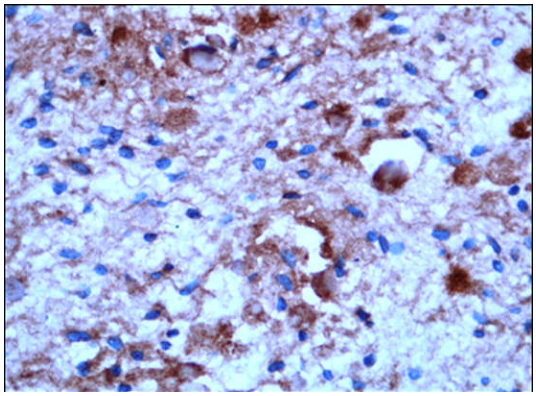

Figure 7. Podoplanin expression (1+) in areas of rosenthal fibre and eosinophilic granular bodies.

Low grade gliomas did not show podoplanin expression. Similarly the study done by J Riedl et al. (2017)[13] who studied 213 gliomas including 185 GBM and 28 anaplastic glioma. Out of 213 tumour specimens, 151 tumour positive for podoplanin (71 low, 47 medium and 33 high expression). Podoplanin expression was positively correlated to grade of intravascular platelet clusters ($p<0.001$). Similarly results also found in study done by Mir Seyed Nazari P et al. (2018)[14] who studied 213 glioma including 152 GBM, 38 anaplastic astrocytoma,10 low grade and 13 others tumous. They found that mostly GBM (82%) showed a strong expression while mostly low grade gliomas showed weak or no expression of podoplanin. Similarly observations also found in study done by Birner P et al. (2014)[15] who studied 354 gliomas including 72 WHO grade II tumours, 32 WHO grade III tumours and 250 WHO grade IV tumours. Out of 354 gliomas, 129 gliomas were positive for podoplanin (48% WHO grade IV, 18.8% WHO grade III and 4.2% WHO grade II). The negative/ low expression of podoplanin in some glioblastoma cases suggests that other associated factors are needed to enhanced clonal expansion of neoplastic cells. Thus the précised role of podoplanin in tumour development is still not fully understood. It is suggested that podoplanin promote tumour invasion through re-organization of cytoskeleton of neoplastic cells not only by combined cell migration, but also by individual cell migration after loss of E-Cadherin and thus play role in tumourogenesis[10]. Podoplanin exhibited two patterns of expression in variable grades ie diffuse and peripheral pattern. In peripheral staining pattern, only the peripheral cells of the tumour island exhibited podoplanin positivity. On contrast in diffuse pattern most of the neoplastic cells in the island displayed podoplanin positivity. This type of pattern also reported in some tumour other than glioma. Stefan J et al (2015)[12] in their study expression was focused on areas of high angiogenic activity with podoplanin positive cells scaffolding vascular structure considering the importance of podoplanin as a lymphatic marker, these findings might suggest a potential association of PDPN with glioma angiogenesis. Similar findings are also present in our study. In our study we found that Podoplanin shows strong positivity in areas of rosenthal fibres and eosinophilic granular bodies in pilocytic astrocytoma. But in other studies this type of observation was not seen.

Association of Ki-67 with Grade of glioma: In our study the majority of cases with Ki-67 score 1 (<4% Li) were of low grade gliomas, while cases with Ki-67 score 2 (4-10% Li) have majority of cases of high grade glioma in comparision to low grade glioma. The cases with Ki-67 score 3 (>10% Li) were all in high grade gliomas. These data indicate that Ki-67 score correlates with the grade of tumour ($p<0.001$). Thus, Ki-67/MIB-1 is useful for differentiating between high and low-grade gliomas, but categorizing score 4-10% is more problematic due to the overlap of values between the different tumor grades. This overlap is a main limitation of this immunostaining. Our study is in concordance with the study of Anne J Skjulsvik et al. (2014)[16] who have taken 267 gliomas cases in which 89 (33.3%) cases were of glioblastomas. They found that the Ki-67/MIB-1 PI correlated significantly with tumor grade for each glioma type. However, considerable overlap was observed between the malignancy groups which is in concurrence with our study. There was no significant difference in Ki-67 PI between glioma type of the same tumour grade. However anaplastic oligodendrogliomas and anaplastic oligoastrocytomas had indices comparable to glioblastomas. They found that indices for high-grade gliomas (grade III/IV) were significantly higher than in low-grade (grade I/II) tumors and concluded that Ki-67 is useful for differentiating between high and low grade glioma. Our results are also in concordance with the study done by Xinhua Hu et al. (2013)[17] who found that the mean Ki-67 LI significantly increased with the glioma grade. Significant difference was identified in the Ki-67 LI between the various glioma grades ($P<0.05$), suggesting that the pathological grade was associated with the Ki-67 LI.Similar results were found in the study done by Andreas H Habberstad et al. (2011)[18] who determined proliferative activity in 27 cases of astrocytomas in which the proliferative activity determined by Ki-67 positively correlated to tumour grade.

The Ki-67 LI displayed a wide range of values that overlap with indices in both grade II and IV astrocytomas. Thus they concluded that this marker should not be used alone but should be used in combination with established histopathological criteria of malignancy.

Association of Podoplanin and Ki-67: In our study majority of patients had podo score 1 having Ki-67 score 1 (75.9%) while majority of cases had podo score 2 having Ki-67 score 2 (66.7%) and Ki-67 score 3 (81.1%). Association of Ki-67 score and podo score was found to be statistically significant ($p<0.001$). To the best of our knowledge there is no relevant data provided by research articles correlating the association of podoplanin and Ki67 in gliomas. Other than glioma only one research article found i.e.Yokota K et al. (2013)[19]they analysed expression of podoplanin and Ki-67 in small sized thymoma and found that stage IVb thymoma have high ki67 index and positive for podoplanin as compared to low grade thymoma.

Comparison of Survival with the histological grading: In our study out of 150 glioma cases only 117 were included in survival data analysis. The rest 33 cases were excluded as 22 (14.7%) cases were lost to follow up and 11 patients died due to post- op complication. The mean survival time of the patients of high grade glioma was significantly reduced in comparison to the patients of low grade gliomas. Difference in mean survival duration of low grade glioma and high grade glioma cases was found to be statistically highly significant ($p<0.001$). Our results are in concordance with the study done by TadejStrojnik et al (1999)[20] in which they found that the rate of death was much lower in the group of benign tumors (45%; *i.e.,* 12 patients) compared with the group of malignant tumors (90.5%; *i.e.,* 66 patients), whereas 93% (54 patients) died within the group of patients with glioblastoma multiforme. A high histological score was highly significant ($P=0.0001$) for poor prognosis. Our results are also in concordance with the study of David W Ellison et al. (1995)[21] who studied 123 adult cerebral astrocytic tumoursand found that the most important prognostic indicator was type of astrocytic tumour. Anaplastic transformation of an astrocytoma or a diagnosis of glioblastoma significantly ($P < 0.0001$) shortened survival, and this effect was independent of other variables.

Comparison of survival with podoplanin score: We found that patients having lower podo score (0-2) have higher mean survival than the patients with higher podo score (3-4). This difference was found to be statistically significant. Similarly study done by Riedl J et al (2018)[13] found that podoplanin expression was associated with increased risk of overall mortality during the 2 year follow up period. In Kaplan- Meier analysis, probability of survival after 6, 12 and 24 months was 98.4%, 88.2% and 68% respectively in patients with Podoplanin negative tumors, 81.5%, 61.4% and 39% for low podoplanin expression and 72.2%, 49.3% and 21.9% for high podoplanin expression.

Comparison of survival with Ki67 score: Proportion of expiry is higher among high grade gliomas as compared to that in low grade gliomas in all the Ki-67 score in this study. Majority of patients survived with score 1 of 1 Ki-67, while majority of the cases were expired with a score 2 and 3 of Ki-67. This shows that with increasing Ki-67 score the survival of patients decreases and that there is a negative correlation between Ki-67 score and survival. However our study also proves that survival not only depends on Ki67 score but also on the histological grade. Hence Ki-67 alone cannot be used as a single prognostic marker. Our study is in concordance with the study done by Tarik Tihan et al (2000)[22] They analyzed the Ki-67 (MIB-1) labeling indexes in the stereotactic biopsy specimens from 11 pilocytic astrocytomas; 8 grade 2, 15 grade 3, and 16 grade 4 diffuse astrocytomas. The tumour with low Ki-67 Li (15%) had better survival then tumours with high Ki67, and concluded that there was a strong correlation with poor outcome when Ki67 LI were higher than 15% in the same tumor for diffuse astrocytoma. Similar results were found in the study done by Fisher BJ et al (2002)[23] who evaluated the expression of Ki-67 in 180 low-grade glioma tumor specimens

immunohistochemically and correlated its expression with prognosis or tumour recurrence. They found that an average Ki-67 value of ≥5% was prognostically significant for reduced cause-specific survival (CSS, p = 0.05) and a Ki-67 level ≥10% was strongly significant of a poor survival outcome (p = 0.009). They concluded that Ki-67 is a useful predictor of CSS in low-grade gliomas; however, it is not independent of other prognostic factors, particularly age.

Survival analysis for radiotherapy: Out of 117 cases, 22 cases who received radiotherapy only 1(1/22) expired, while among 95 cases who did not receive radiotherapy, 59 (64.1%) expired. Mean survival duration of cases who received radiotherapy was significantly higher as compared to those who did not receive radiotherapy.

CONCLUSION

The study concludes that the survival rate is usually low in case of high grade gliomas and Podoplanin scoring and Ki67 scoring increased with increasing grade. The study correlates the expression of Podoplanin with the increasing grade of glioma hence providing information about its use as a future prognostic marker, also the inhibitors of podoplanin could be used as a potent strategy for treating cancer as it's upregulation is seen in malignancies.

Conflict of interest: Nil

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