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Prostate Specific Membrane Antigen Expression in Neovasculature Associated with Glioblastoma Multiforme and Other Astrocytic Neoplasms; Immunohisto Chemical and Histopathological Study

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Abstract: Glioblastoma (GB) is the most frequent malignant brain tumor and it is a highly vascularized one. PSMA represents a unique endothelial target as it has been identified in the endothelial lining of the solid malignant tumors neovasculature, but not within the normal ones. The aim of this study was the detection of expression & evaluation of intensity of PSMA on the neovasculature of GB and some of other astrocytic neoplasms versus normal brain vasculature. Serial sections of 56 paraffin embedded blocks of GB and other astrocytic tumors were immune stained with CD34 antibody for identification of vascular structures and endothelial proliferation and with PSMA antibody for assessment of endothelial cell uptake in the vasculature of both normal brain tissue and the astrocytic tumors associated neovasculture. Results showed the following positive immunohistochemical staining for CD34 was detected in all vessels (Normal and tumor associated). Tumor associated marked vascular proliferation with or without endothelial proliferation was detected in (75%) of all cases and it showed a statistically significant relationship in GB cases versus Non-GB astrocytic tumors (P-value <0.001). PSMA expression was detected within endothelium of tumor associated neovasculature (77% of cases) and not within normal brain endothelium. It was significantly detected in GB versus Non GB other astrocytic tumors (P-value =0.044).Conclusions: PSMA positive expression was detected in a significant proportion of the endothelial cells of high grade astrocytic tumors, while its expression in low grade ones was not clearly established and need thorough future studies concerned mainly with low grade astrocytoma.

Key words: GB · Vascular and Endothelial Proliferation · PSMA

INTRODUCTION

Glioblastoma is the most frequent malignant brain tumor [1] but its true incidence in Egypt is quite unclear [2].Despite progression in therapy, the survival remains extremely poor [3].GB is one of the most vascularized tumors in humans [4].Other astrocytic tumors may show vascular proliferation such as Pilocytic astrocytoma [5]. Anaplastic astrocytoma may also show hypertrophy of the lining endothelium [6].

The vascular endothelium is much more susceptible to the antibody than the tumor substance, which is protected by the blood brain barrier [7] and the tumor vasculature differs significantly from normal vasculature, which may represent a hopeful target for anti-angiogenic tumor therapy [8].

PSMA is a highly specific type II membrane protein, it has been identified in the vasculature associated with solid malignant tumors, but not within the normal endothelium [9].Some studies have detected its presence in the vasculature of GB vessels as well Wernicke *et al.*[10].It is rapidly internalized so it is suitable target for anti-angiogenic therapy [11].PSMA was first thought to be prostate restricted [12] but later studies demonstrated that it may be also expressed by small intestine, renal tubules, brain and salivary glands [13] in which the expression is 100–1000-fold less than in prostate tissue and the site of expression is not accessible for circulating antibodies [14].Clinical trials on anti-PSMA antibody (J591) based anti-angiogenic therapy has promising results, they were successfully used targeting the neovasculature of some non prostate malignancies

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in vivo [15, 16] which may represent a future hope for therapy in such refractory malignant brain tumors.

Aim of the Work: The detection & evaluation of intensity of PSMA antigen expression on the neovasculature of glioblastoma and other astrocytic neoplasms.

MATERIAL AND METHODS

Study Group: Fifty six paraffin embedded tissue blocks; that were obtained from Kasr el-aini hospital, diagnosed during the last decade.

Histopathological Evaluation: Three sequential serial sections of 4 -μthickness were cut from the formalin fixed paraffin embedded tumor blocks, stained by *H&E* for histopathological evaluation according to WHO 2007 criteria, *CD 34*antibody for evaluation of vascular structures, lastly by *PSMA* antibody for assessment of endothelial cells uptake in both normal and malignant associated neo-vasculature.

Immunohistochemical Procedure: Immunohistochemical reaction were carried out using Avidin-Biotin immunoperoxidase system by using PSMA monoclonal antibody(Clone SP29) manufactured by Neomarkers for Lab Vision Corporation (Rabbit Ig G) diluted at 1:50 and pre-diluted CD34 monoclonal antibody for endothelial cells (clone QBEnd\10) (Mouse Ig G), manufactured by ScyTek Laboratories.

Procedure: Sections of 4 ìm in thickness were deparaffinized in xylene and rehydrated through a series of graded alcohols. Antigen retrieval of the sections was done. Heating tissue sections in 1mM EDTA, PH 8.0, for 10-20 minutes, 98 degree followed by cooling at room temperature for 20 minutes was done for this purpose. The sections were incubated with anti-PSMA monoclonal antibody diluted 1:50 in phosphate buffer and pre-diluted anti-CD34, for 60 minutes and then incubated in enzyme conjugate for 10 minutes. The reaction was visualized with the Zymedimmunohistochemical detection kit using diaminobenzidinechromogene as substrate. Finally, they were counterstained with Mayer's hematoxylin.

Control: In each staining session, a section of prostatic carcinoma known to be positive for PSMA was used as positive control and a section of skin tissue served as positive control for CD34.

As a negative control, a tumor tissue section was processed in the above mentioned sequence but the primary antibody was not added and instead PBS was used in this step.

Immunostaining Interpretation: CD34 immunostained sections were assessed for the vascular structures in normal brain and in tumors, for endothelial cells presence and proliferation (*Brown staining was localized in the cell membrane of endothelial cells),* then PSMA immunostained sections were assessed for endothelial uptake &percentofintensityof staining in normal brain and tumor vasculature *(Brown staining was localized in the cytoplasm or cell membrane of endothelial cells).*

Extent of vascular endothelial staining (Percent staining) for PSMA was estimated as follows:

- Less than 5% of vessels staining (None)
- 6% to 25% of vessels staining (Minimal)
- 26% to 50% of vessels staining (Moderate)
- 51% to 75% of vessels staining (Strong)
- 76%-100% of vessels staining(Very strong)[10].

Statistical Method: Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21.

*Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between the 2 groups with respect to normally distributed numeric variables were done using the t-test. For categorical variables, differences were analyzed with 2 (Chi square) test and Fisher's exact test when appropriate. All p-values are two-sided. P-values < 0.05 were considered significant

RESULTS

The study was composed of 56 cases; 42 cases grade VI; GB(75%), 4 cases grade III; anaplastic astrocytoma (7%), 5 cases grade II;2 as diffuse fibrillary astrocytoma (4%) and 3 as pleomorphic xanthoastrocytoma(5%), &5 cases grade I; 4 as pilocytic astrocytoma (7%)and one as subependymal giant cell astrocytoma (2%).

PSMA expression was significantly expressed in the endothelium of the malignant associated neovasculature of GB (81% of positive astrocytic tumors) versus 19% in Non-GB astrocytic tumors (19% of positive astrocytic tumors). P-value =0.044(Total No =43)

Table 1: Age distribution in astrocytic tumors:

	Glioblastoma		Non-glioblastoma	
Age group	Number	Percentage%	Number	Percentage%
0-<15 y	2	5	6	43
15-<20	2	5	1	7
20-<25	1	2	0	0
25-<30	2	5	2	15
30-<35	3	7	0	0
35-<40	2	5	1	7
40-<45	1	2	1	7
45-<50	5	12	0	0
50-<55	6	14	2	14
55-<60	7	17	1	7
60-<65	5	12	-	-
>65	4	9	-	-
Unknown	2	5	-	-
Total	42	100	14	100

Table 2: Gender distribution inastrocytic tumors

	Glioblastoma		Non-glioblastoma	
Gender	Number	Percentage%	Number	Percentage%
Male	30	71.4	8	60%
Female	12	28.6	6	40%
Total	42	100	14	100

Table 3: Anatomic site distribution in astrocytic tumors

Site	Number	Percentage%
cerebral lobes	47	83.9
cerebellar	3	5.4
Ventricular	2	3.6
Other sites	4	7.1
Total	56	100

Table 4: The frequency of cerebral lobe affection in astrocytic tumors

Site	Number	Percentage%
Frontal lobe	16	34
Parietal lobe	18	38.3
Temporal lobe	12	25.5
Occipital lobe	1	2.1
Total	47	100

Table 5: Count of lesions in astrocytic tumors

Count	Number	Percentage%
Single site	39	69.6
Two -multiple sites	17	30.4
Total	56	100

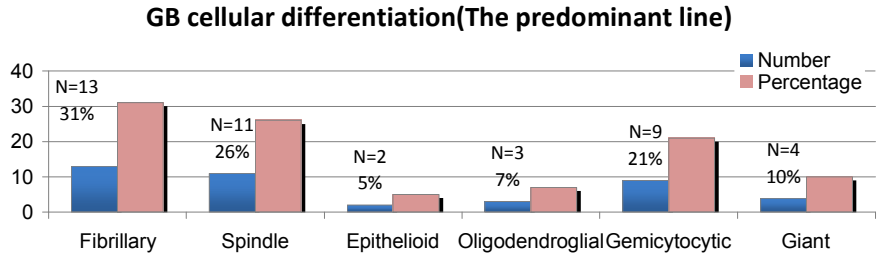

Chart 1: GB cellular differentiation (The predominant line)

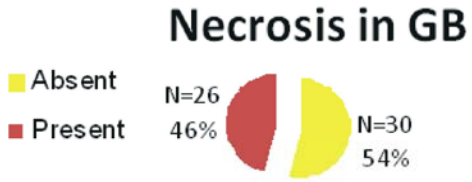

Chart 2: Presence of necrosis in GB.

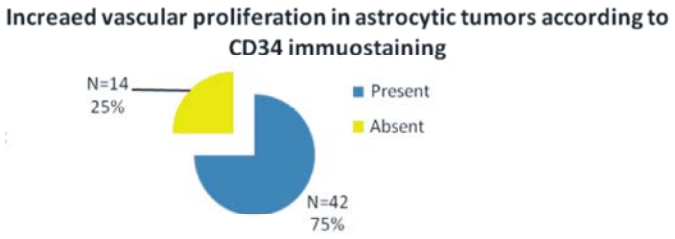

Chart 3: Increased vascular proliferation according to CD34 immunostaining in astrocytic tumors

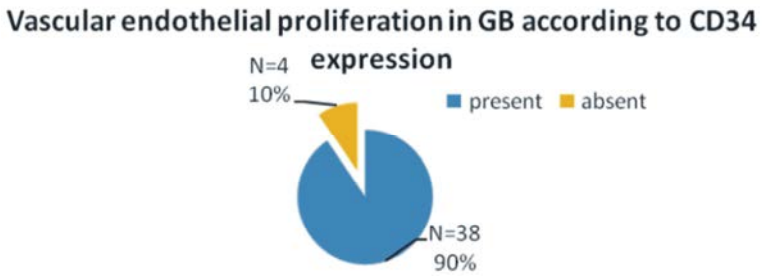

Chart 4. Vascular endothelial proliferation in GB according to CD34immunostaining

Table 6: Increased vascular proliferation in GB versus Non-GB astrocytic tumors according to CD34 expression:

Vascular proliferation	Number	Percentage%
GB	38	90
Non-GB	4	10
Total	42	100

Table 7. PSMA expression in astrocytic tumors

Expression	Number	Percentage%
Positive	43	77
Negative	13	23
Total	56	100

Table 8: PSMA expression in GB:

Expression	Number	Percentage%
Positive	35	83
Negative	7	17
Total	42	100

Table 9: PSMA expression in Non-GB astrocytic tumors:

Expression	Number	Percentage%
Positive	8	56
Negative	6	43
Total	14	100

Table 10: PSMA expression in GB versus Non-GB astrocytic tumors:

PSMA positivity	Number	Percentage%
Glioblastoma	35	81
Non-GB	8	19
Total	43	100

Table 11: Extent of vessels staining by PSMA in astrocytic neovasculature:

	Glioblastoma		Non-glioblastoma	
Percentage	Number	Percentage%	Number	Percentage%
Less than 5%	7	17	6	43
5%-25%	7	17	1	7
>25%-50%	4	9	1	7
>50%-75%	12	28	4	29
>75%-100%	12	29	2	14
Total	42	100	14	100

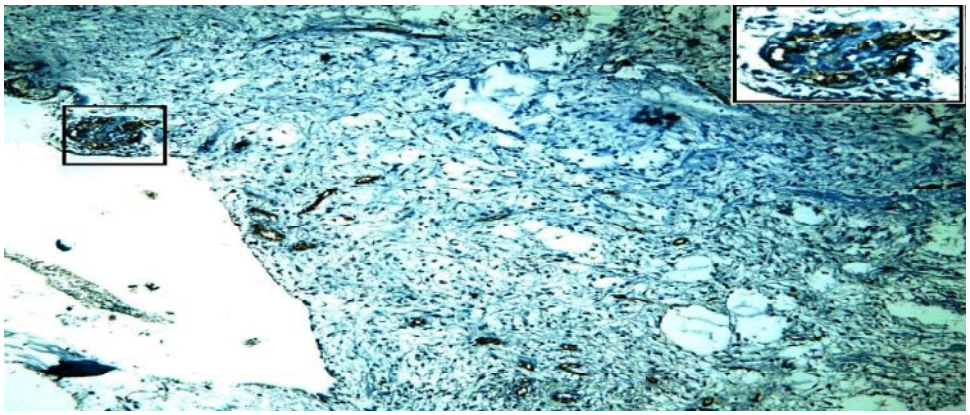

Fig. 1: Pilocytic astrocytoma with glomeruloid vasculature positively immunostained by CD34. Original magnification power 100x, inset 200x.

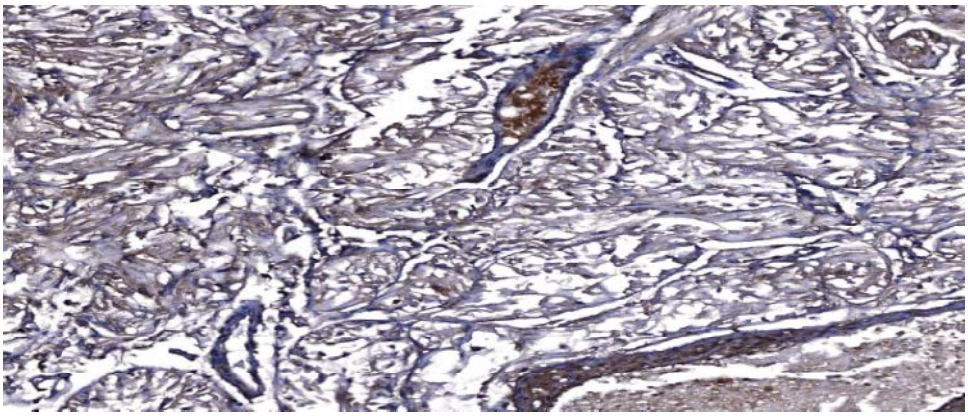

Fig. 2: Pilocytic astrocytoma showing PSMA positive expression of endothelial lining of the blood vessels. Original magnification power 200x.

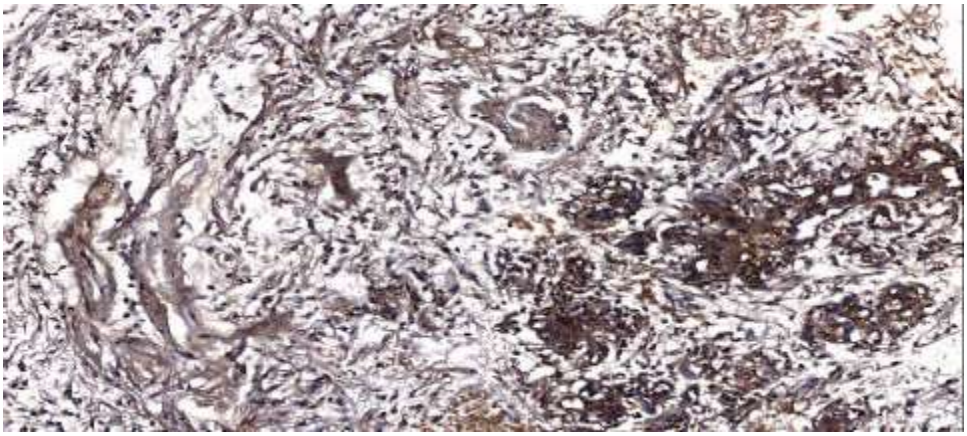

Fig. 3: Pilocytic astrocytoma showing glomeruloid vasculature positively stained with PSMA(Cytoplasmic and membranous). Original magnification power 200x.

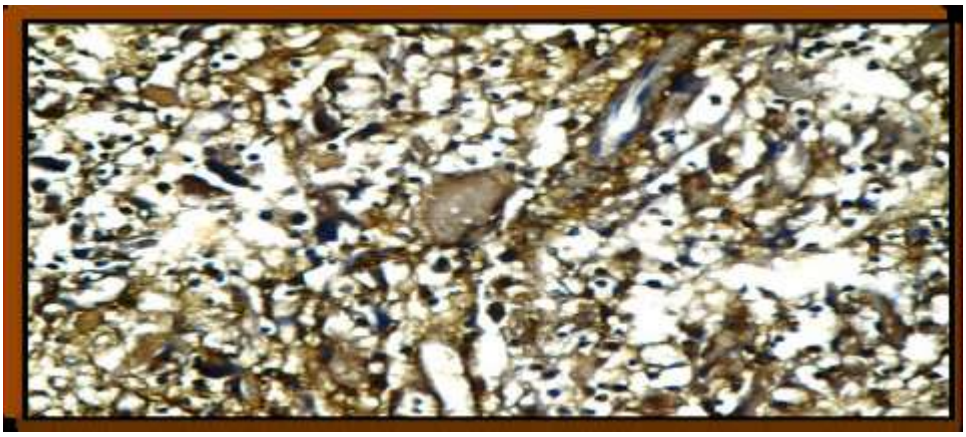

Fig. 4: PXA showing positive endothelial cytolasmic and membranous expression of PSMA. Original magnification power 200x.

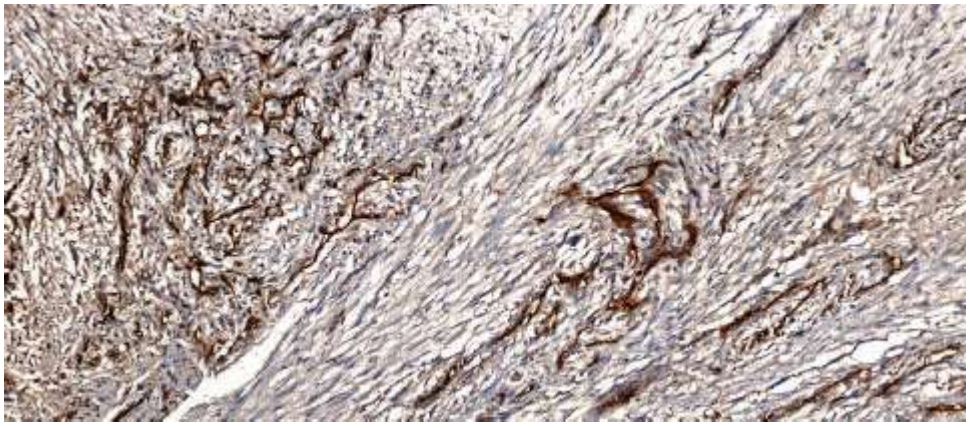

Fig. 5: GB with sarcomatoid features showing membranous expression of PSMA of the endothelial lined vascular spaces.Original magnification power 200x.

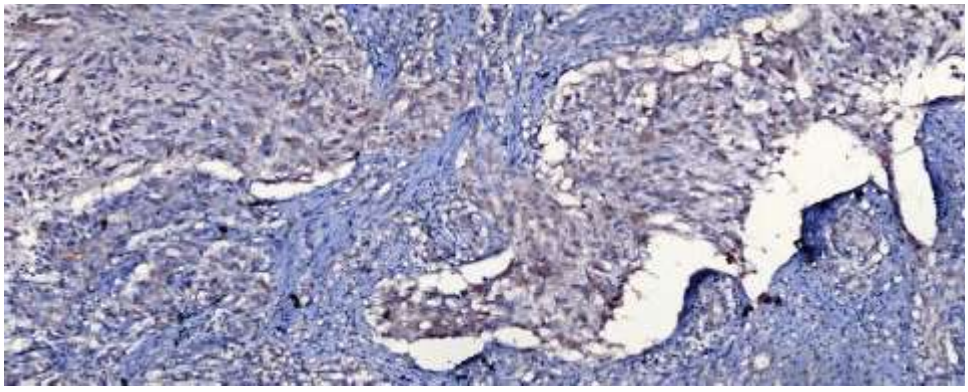

Fig. 6: GB showing marked endothelial proliferation, with cytoplasmic expression of the endothelial cells, Original magnification power 100x

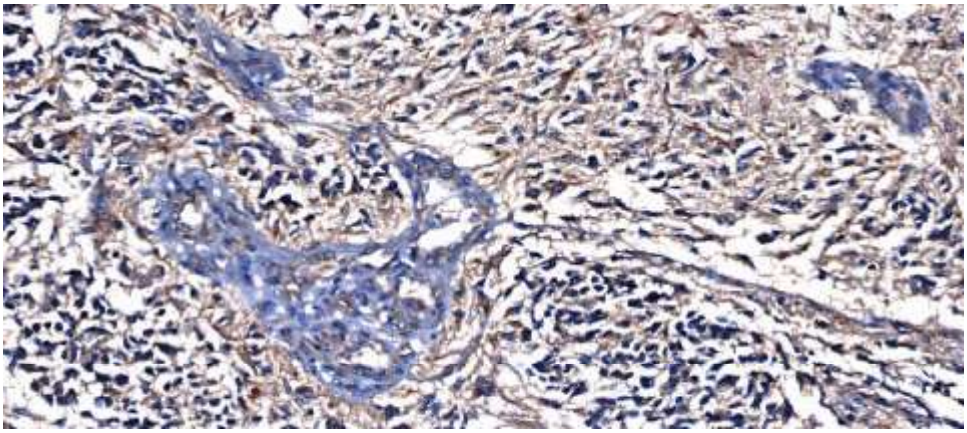

Fig. 7: GB showing glomeruloid body formation, with cytoplasmic and membranous endothelial cells PSMA expression. Original magnification power 200x

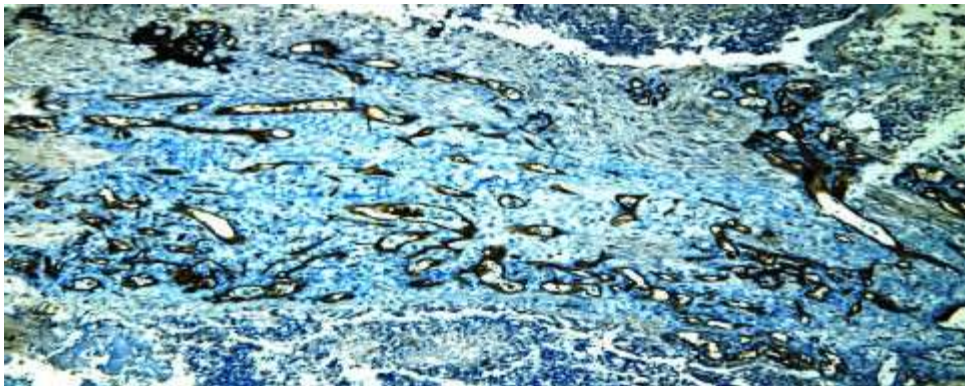

Fig. 8: GB with increased vascular proliferation as evaluated by CD34 positive immunostaining. Original magnification power 100x.

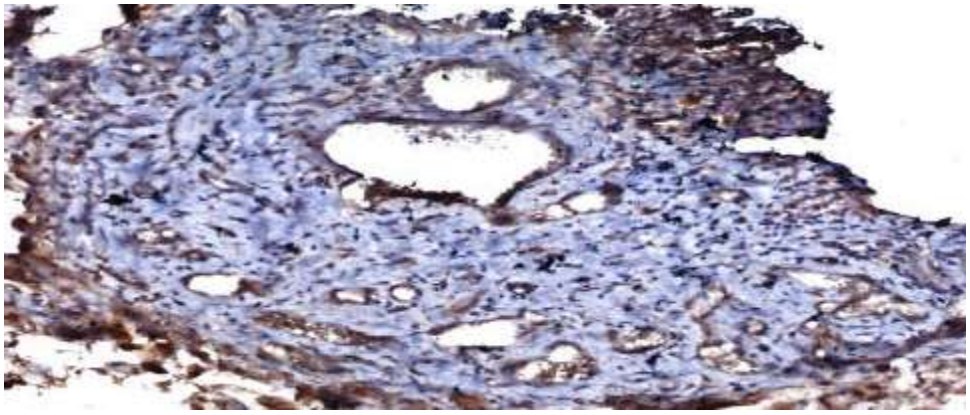

Fig. 9: GB showing glomeruloid endothelial proliferation, with cytoplasmic and membranous endothelial cells expression of PSMA, Original magnification power 200x

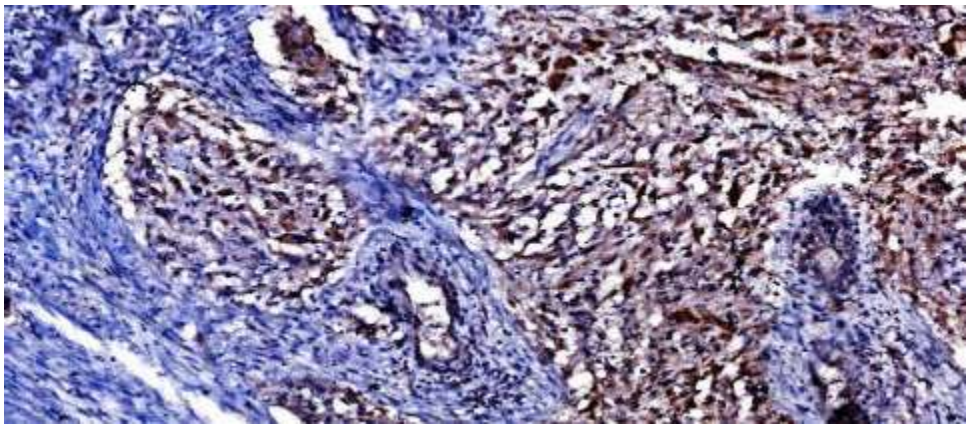

Fig. 10: GB showing marked endothelial vascular proliferation, with positive PSMA staining of the endothelial cells. Original magnification power 200x.

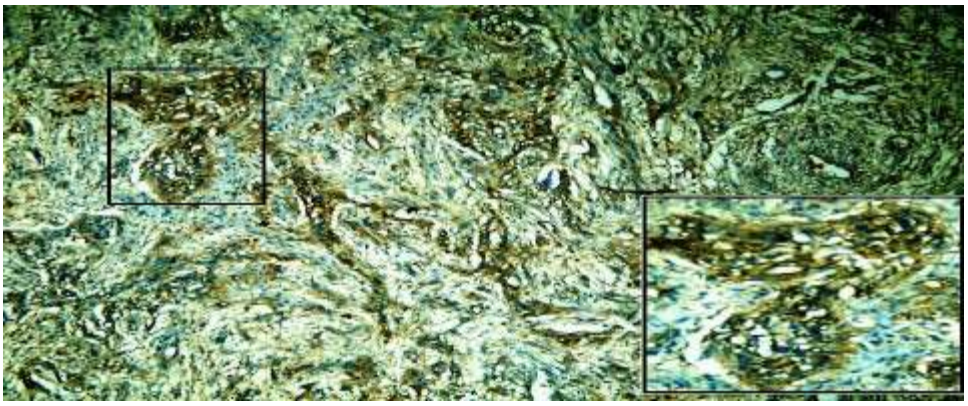

Fig. 11: GB with endothelial vascular proliferation and glomeruloid body formation, the endothelial cells showed cytoplasmic and membranous PSMA immunostaining. Original magnification power 100x. Inset 200x.

DISCUSSION

Malignant glioma remains an interactable problem for therapy. Although systemic metastasis are relatively rare,only 5% or less will survive for 5 years after diagnosis. The tumor isextrememly aggressive in a locally invasive fashion [17].

In the current study The most commonly affected age group in GB was (50->60 years) representing (31%), which is in concordance with a study was performed in Mansoura university screening the East Delta, where it was (50->60 years) and representing 33.5% [18].

The median age for GB in the present study was 51 years which is approaching that obtained in Italy, where it was 58 years[19]and that of Kanno *et al*.[20]where it was *57 years*, but itis less than that obtained within the results of the Middle East Cancer Consortium beginning in the year 1999 among the residents of Gharbiah which was 64 years [21]and that ofUSA by Betsy*et al.*[22]which also was 64 years. This may be explained by that the risk to develop gliomainreases with age, racial factors and bigger sample size.

In this study GB occurred more commonly in males representing 71%, male: female ratio was 2.1:1 which was consistent with but with higher male incidence than that of Kenneth *et al.*[23]where male to female ratio was 57.6%: 42.2 (1.3) and those of Filippini *et al.* and Betsy *et al*. [19, 22]who both stated that it is 1.6 times more frequently in males than in females.Higher male incidence was explained by some investigators that female sex hormones have a protective effect against brain cancer, while others have suggested innate differences in the susceptibility of X and Y chromosomes to tumorigenic stimuli [24].

In the current study the cerebral lobes were the most frequently affected site representing 83% which is in agreement with a study done on Finnish citizens where most of the gliomas were located in the cerebral lobes (86%)[25]and CBTRUS Statistical Report during 2004-2008 which stated that the collective sum of affection of the cerebral lobes was 60% [26].

In this study the parietal lobe was the most commonly affected site representing 38%, followed by the frontal representing 34%, which is rather different from the Finnish study data which stated that gliomas incidence in the frontal lobe accounted for 40%, temporal lobe for 29%, parietal lobe for 14% and occipital lobe for 3.0% of the cases [25]and CBTRUS Statistical Report which stated that the Frontal Lobe was 25.3%, Temporal Lobe19.6%, Parietal Lobe12.7% [26]and approaching Niloofar*et al.*[27] as the primary site was Frontal in (32%), Temporoparietal in (49%), which may be explained by difference in sample size.

In the current study glioma was predominantly confined to single site accounting for 87.5% of the studied material while those which developed in two or multiple sites accounted for 12.5%, which is similar with lesser ratios than those obtained by Larjavaara *et al.*[25]who stated that (68%) of their sample size were located in only one site, whereas (32%) were overlapping two or more sites while Filippini *et al.* [19] found that a single lobe accounted for 57% of the studied cases.

In the current study endothelial vascular proliferation was detected with variable grades, from doubling of the endothelial layer up to glomeruloid body formation in 90%, which is less than that obtained from

Homma *et al.*[28] who reported vascular proliferation in 100% of their studied material and also that of Aamir and Anwar Ul-Haque [29]who also detected it in 100% of cases.

In the present study; ischemic and/or pseudopalisading tumor necrosis in GB was detected in 46% which is less than detected by Aamir and Anwar Ul-Haqu[29]as pseudopalisaded necrosis was present in 60% of their cases and Homma *et al.*[28] where large ischemic and/or pseudopalisading necrosis was observed in (87%) of cases.

The predominant patterns of GB cellular differentiation in this study, fibrillary(31%), spindle(26%), gemicytocytic(21%), epithlioid(5%), oligodendroglial(7%) and giant cell(10%), which is partly similar to that documented by Popov *et al.*[30]where the predominant subtype was fibrillary (53.6%), gemistocytic (7.9%), giant cell (3.9%), small cell (6.9%), oligodendroglial (21.7%) and sarcomatous (5.8%).

In the current study PSMA expression in GB neovasculature was not detected in normal brain microvessels which were present in16 of cases (Internal control cases) and detected in malignant associated vasculature of 83% of cases, 57% had more than 51% staining and 40% in each of grade I and II and 100% of Grade III vessels. These results are less than a study done by Wernicke *et al.*[10]where it was expressed in (100%) of cases with variable extent; (69%) had more than 51% vascular staining for PSMA, this difference may be attributed to different PSMA subclones used.

It was somewhat different from a study done by Natsuko *et al*. [32] who used 4 autopsies for demonstrating normal brain microvessels as a control cases and they did not stain positive for PSMA. Tumor microvessels of grade IV glioma showed intense PSMA staining. Grade I; moderate, while grade II and III; no vessel staining, but a few (<2%) of the tumor cells stained.

CONCLUSIONS

PSMA positive expression was detected in a significant proportion of the endothelial cells of high grade astrocytic tumors, which may be of importance in anti-angiogenic therapy, while its expression in low grade ones was not clearly established in the current study and need thorough future studies concerned mainly with low grade astrocytoma.

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