RESEARCH ARTICLE

Editorial Process: Submission:02/02/2023 Acceptance:05/18/2023

Prostate-Specific Membrane Antigen (PSMA) Expression in The Neovasculature of High Grade Gliomas (Histopathological and Immunohistochemical Study)

Mona Salah-Eldin Abdel Maguid, Menna-t-Allah Gamal Saad El Dine*, Samia Mohamed Gabal, Sara Mahamad Abou Fandoud

Abstract

Background: Prostate-specific membrane antigen (PSMA) was first noticed in prostate cancer cells, thereafter, It has been found in the endothelial cells of neovasculature in a variety of tumors, but not in normal vascular endothelium, This specificity makes PSMA an ideal molecule for vascular targeting in Cancer theranostics (i.e., combined diagnostic and therapeutic). **Objectives:** The objective of this study was to evaluate the immunohistochemical (IHC) expression of PSMA in the neovasculature (identified by CD 31) of high-grade gliomas (HGGs) and to Correlate PSMA IHC expression in HGGs with clinicopathological features, to detect its possible role in tumor angiogenesis, where PSMA can be used as a future diagnostic and therapeutic target. **Materials and Methods:** This retrospective study included a total of 69 archived, formalin-fixed, paraffin-embedded tissue blocks of HGGs, including 52 cases classified as WHO grade IV (75.4%) and 17 cases as WHO grade III (24.6%). The samples were immunohistochemically analyzed for PSMA expression (in both TMV and parenchymal tumor cells) which was assessed using the composite PSMA immunostaining score. A score (0) was considered negative while scores 1-7 were considered positive (1–4, 5–6, or 7; weak, moderate, or strong respectively). **Results:** PSMA is expressed specifically and significantly in endothelial cells of tumor microvessels (TMV) of HGGs, A statistically significant relationship was detected between PSMA IHC expression in both TMV and in parenchymal tumor cells (TC) and various glioma subtypes (P-value < 0.05 and <0.001 respectively). Positive PSMA immunostaining in TMV was detected in all anaplastic ependymoma cases and in near all cases of classic GB and GB with oligodendroglial features more than other subtypes, with P-value specifically for PSMA positivity/negativity in TMV statistically significant (0.022). While for Tumor cells, Positive PSMA immunostaining was detected in all anaplastic ependymoma, most anaplastic astrocytoma and classic GB cases in contrary to other variants, with P-value statistically extremely significant (< 0.001). Comparing PSMA IHC expression in TMV and its expression in TC, it was significantly expressed in TMV of 82.7% versus TC of 51.9% of grade IV cases. Likewise, in GB with oligodendroglial features and gliosarcoma, the majority of cases showed positive staining in their TMV [8/8 (100%), 9/13 (69.2%) respectively], and, the reverse occurs in tumor cells where the majority of cases did NOT show staining in the tumor cells for PSMA (5/8 (62.5%), 11/13 (84.6%) of cases respectively), which was statistically significant (P-value ≤ 0.05) besides the significant difference in the pattern of staining according to composite PSMA scoring (P-value ≤ 0.05). **Conclusion:** PSMA has a possible role in tumor angiogenesis, therefore it might be considered a potential promising endothelial target for Cancer theranostics with PSMA-based agents, in addition, PSMA was expressed significantly in TC of HGGs, thus, it appears to be involved in biologic behavior, carcinogenesis and tumor progression.

Keywords: Gliomas- high grade- glioblastoma- angiogenesis- neovasculature- tumor microvessels

Asian Pac J Cancer Prev, **24 (5)**, 1797-1808

Introduction

High grade gliomas are the most aggressive and deadly types of brain tumors and make up the vast majority of all malignant primary CNS neoplasms, with glioblastoma (GB) being on top of all malignant primary CNS tumors and gliomas (49.1% and 58.4% respectively) (Ostrom et al., 2021). Gliomas make up about a third of all primary CNS tumors in Egypt, with astrocytic tumors accounting for the most common (79.4% of all gliomas), followed by ependymomas and oligodendrogliomas. The most common glial tumor is GB, which makes up 38.3% of all gliomas, while low-grade diffuse astrocytoma made up only 17.9% (Zalata et al., 2011).

*Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt. *For Correspondence: menna.gamal@kasralainy.edu.eg*

Complete neurosurgical resection of these tumors is impossible due to their infiltrating nature (Juratli et al., 2013). Thus, standard treatments for GB and other HGGs include maximum safe surgical resection, radiation, and chemotherapy to postpone recurrence and increase survival rates (Bush et al., 2017). However, Gliomas have a poor prognosis due to their complex pathogenesis, and current treatments haven't improved overall patient survival rates (Reifenberger et al., 2017).

Angiogenesis is an active process that controls the growth of new blood vessels from an existing vascular bed and is essential for any regenerative mechanisms as well as tumor survival and growth. In the case of solid cancers, there is a pathological imbalance in favor of proangiogenic factors, resulting in abnormal, leaky, novel capillary formation, which is required for rapid growth and metastasis. As a result, pro-angiogenic factors and their receptors are appealing targets in the molecular imaging of tumor vascularisation and viably evaluation (Jayson et al., 2016).

HGGs exhibit high vascularity, and vascular proliferation is a pathological hallmark of GB (Brem et al., 1972). In addition, in brain tumors, the blood-brain barrier (BBB) protects the tumor bulk at least partially, thus the endothelium is much more easily exposed to antibodies than tumor substance (Wernicke et al., 2011). These features suggest that vascular targeting agents may have a great impact as a complementary therapy in HGGs by causing a rapid decrease in tumor blood flow as well as widespread tumor necrosis (Thorpe, 2004).

Many tumor cells produce vascular endothelial growth factor (VEGF), which is an angiogenic factor. Hypoxia stimulates VEGF secretion, which stimulates angiogenesis and leads to increased vascularity (Tsui et al., 2005). Anti-VEGF antibody, bevacizumab, is approved for treatment of recurrent GB by blocking tumor neovascularization, which leads to a reduction in tumor size, but no overall survival benefit has been demonstrated (Wick et al., 2017). VEGF inhibitors have been suggested as a possible combination with anti-PSMA antibodies (Brastianos and Bachelor, 2009). This is due to the fact that PSMA appears to cause VEGF-independent angiogenesis (Grant et al., 2012).

PSMA, a very well target for visualising prostate cancer, is a type II transmembrane glycoprotein that was first discovered in prostate neoplastic cells. PSMA is expressed in only a few normal tissues: the prostate epithelium, kidney proximal tubules, nervous system glial cells, and the small bowel jejunal brush border (Mhawech-Fauceglia et al., 2007). PSMA appears to have a role in angiogenesis in neoplasms as well. PSMA's functional role in angiogenesis is unclear at the moment, nor is it required for tumor associated neovascularization, but it is frequently expressed in the endothelial cells of neovasculature of different tumors, including breast cancer (Wernicke et al., 2014), oral (Haffner et al., 2012), glial tumors especially higher-grade gliomas (Mahzouni and Shavakhi, 2019) and others but not in the corresponding normal vasculature (Chang and Heston, 2002). Furthermore, the presence of an internalization sequence within the protein suggests that PSMA-targeted therapeutics could be internalized via binding. Finally, PSMA's peptidase activities suggest that it may be involved in the processing of a pro-drug directed at tumor cells (Tagawa et al., 2012).

Thus, PSMA has evidenced to be an outstanding target for Cancer theranostics (i.e., a combined diagnostic and therapeutic) target, particularly prostate cancer. As a result, plenty of PSMA-targeting tracers have been developed and (pre)clinically tested, with encouraging results (Ruigrok et al., 2019).

Thus, the aim of this study was to evaluate the IHC expression of PSMA in neovasculature (identified by CD 31) of HGGs to investigate its potential role as a diagnostic and therapeutic target to detect its possible role in tumor angiogenesis. and correlate between its expression in high-grade gliomas with available clinicopathological features of such tumors.

Materials and Methods

This is a retrospective cross sectional analytical study that included 69 cases of HGGs "52 cases classified as WHO grade IV (75.4%) and 17 cases as WHO grade III (24.6%)" through collection of archived paraffin blocks of HGGs, from the Pathology Department, Faculty of Medicine, Cairo University during the period from January 2015 till January 2020.

Inclusion criteria: cases diagnosed as HGGs with sufficient material in paraffin blocks for IHC, And the exclusion criteria; improperly fixed specimens, tiny biopsies, and biopsies with extensive hemorrhage & necrosis without viable tumor cells. The medical records were revised, and clinical data were collected as age, gender, tumor site, histological subtype & grade of HGGs. The study took the approval of the ethical committee of the Faculty of Medicine, Cairo University (N-205-2020).

Each paraffin block was cut by rotator microtome at four microns thickness and then mounted on glass slides to be stained with Hematoxylin and Eosin (H&E) for histopathological re-evaluation. Histopathologic examination of H&E stained slides was performed under low power then high power for confirmation of histological subtype and grade of HGGs according to WHO classification of tumors of CNS tumors 2007 (Louis et al., 2007).

Immunohistochemistry

Immunostaining was done using mouse monoclonal antibody targeting PSMA, Catalogue Number (No): abx414664, obtained from Abbexa Ltd, Cambridge, UK. The slides were autostained in BenchMark XT (Ventana) autostainer using ultraview DAB Detection Kit Counterstaining with Hematoxylin. A section of benign hyperplastic prostatic tissue was utilized as a positive control. While for CD31, Immunostaining was done using mouse monoclonal antibody targeting the CD 31 antigen. Catalogue No: abx137037, obtained from Abbexa Ltd, Cambridge, UK. The slides were autostained in BenchMark XT (Ventana) autostainer using ultraview DAB Detection Kit Counterstaining with Hematoxylin.

Evaluation of IHC expression of PSMA

Tumor tissue sections were examined and scored under LEICA ICC50HD microscope at low power then high-power magnification. Expression of PSMA was identified as membranous and/or cytoplasmic staining in TMV and in TC and was accepted as positive. The tissue samples were also stained for CD31 to verify endothelial neo-vasculature localization. The major vessels were not considered. The stained sections were examined for the intensity and the extent of distribution of PSMA immunostaining in endothelial cells of TMV and in TC and scored semi-quantitatively. The IHC reactions were analyzed according to the pattern of staining (Moore et al., 2017).

The intensity of staining was graded on a 0–3 scale as follows: Score 0: absence of staining, score 1: weak staining, Score 2: moderate staining and Score 3: intense staining. The extent of PSMA immunostaining in the examined tumor tissue was subdivided into five categories on the basis of the proportion of immunopositive cells, as follows: Score 0: all tumor cells are negative, Score 1: < 25% of tumor cells are positive, Score 2: 25%– 75% of tumor cells are positive, Score 3: 50%–75% of tumor cells are positive and Score 4: >75% of tumor cells are positive.

The values of the extent of distribution of PSMA immunostaining in endothelial cells of TMV and in TC were added to the average PSMA intensity in each of them respectively, to obtain a composite PSMA staining score for each; endothelial cells of TMV and TC respectively. Score 0 was considered negative when all TC did not show membranous and/or cytoplasmic staining, while scores 1-7 were considered positive (1–4, 5–6, or 7; weak, moderate, or strong respectively) (Matsuda et al., 2018; Saffar et al., 2018). PSMA immunostaining results were correlated with multiple prognostic factors including (tumor site, WHO grade of glioma, and histological subtype).

Statistical Methods

Data were coded and entered into the statistical package for the Social Sciences (SPSS) version 28. Data were summarized using mean, standard deviation, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. For comparing categorical data, Chi-square test was performed. The exact test was used instead when the expected frequency is less than 5 (Chan, 2003). The significance of the results was assessed by determining the probability factor "P-value". P-value ≤0.05 was considered statistically significant.

Results

The sixty-nine cases of HGGs were selected as follows: 52 cases were classified as grade IV (75.4%) and 17 cases as grade III (24.6%). The mean age of all studied cases was 43.54 ± 15.1 years at the time of pathological diagnosis. As regards gender, the males and females were nearly in equal proportion.

From the totally collected HGGs cases, PSMA IHC expression was detected in the endothelial cells of TMV (53/69) (76.8%), and in TC of 39/69 (56.5%) of HGGs cases respectively as shown in Figures 1, 2 & 3. PSMA immunostaining was not observed in normal blood vessels of normal brain tissue, occasionally admixed with our

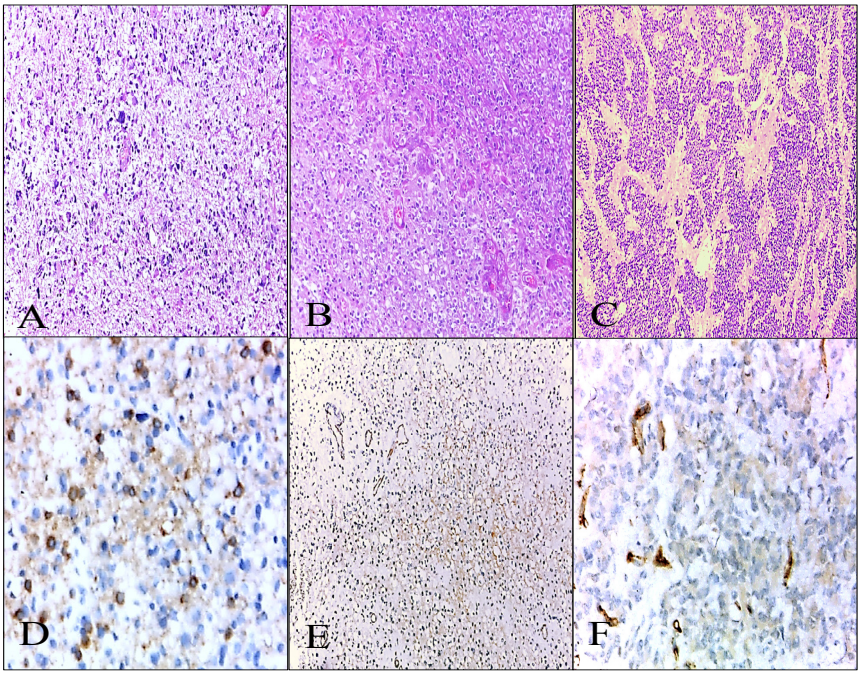

Figure 1. Different Cases of Grade III Gliomas; Figures (A, B &C): Anaplastic astrocytoma (WHO grade III), Anaplastic oligodendroglioma (WHO grade III) and Anaplastic ependymoma (WHO grade III) respectively (H&E, original power x100). Figures (D, E &F): Anaplastic astrocytoma (WHO grade III) shows moderate PSMA expression in tumor cells, Anaplastic oligodendroglioma (WHO grade III) shows strong PSMA expression in TMV, moderate in tumor cells and Anaplastic ependymoma (WHO grade III) shows strong PSMA expression in TMV with moderate expression in tumor cells respectively (IHC, original power Dx400, Ex100 & Fx400).

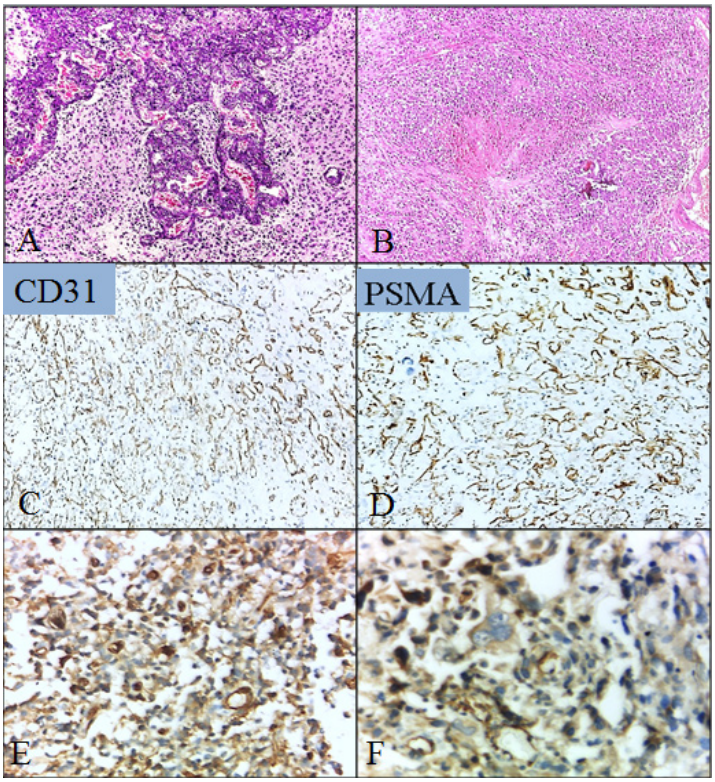

Figure 2. Classic GB (WHO Grade IV). (Figures A&B); MVP and palisading necrosis respectively (H&E, original power x100). (Figures C&D): CD31 and PSMA IHC; strong PSMA expression in TMV with negative expression in tumor cells (IHC, original power x100). (Figures E&F): strong PSMA expression in both TMV and tumor cells (IHC, original power x400).

cases, this specificity makes PSMA an ideal molecule for Cancer theranostics (i.e., combined diagnostic and therapeutic). Even though in the present work, PSMA was expressed by some cells in the normal brain (Figure 4).

PSMA expression in TMV and TC had no statistically significant correlation with any of [gender, tumor site], While for histological grade, the PSMA IHC expression was more positively associated with Grade IV gliomas compared to grade III although didn't rich statistical significance (P=0.054) as shown in (Table 1).

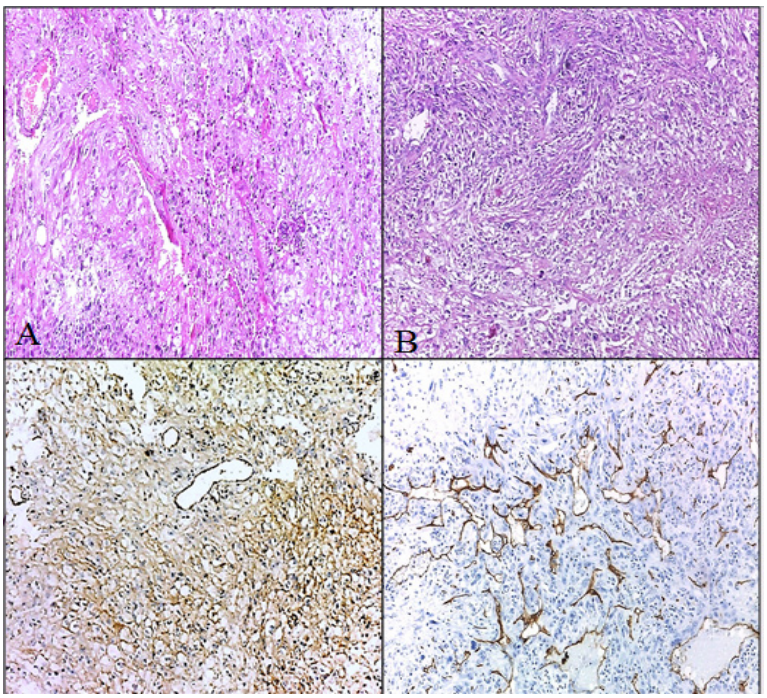

Figure 3. (Figures A&B); GB with oligodendroglial features (WHO grade IV) and Gliosarcoma (WHO grade IV) (H&E, original power x100). (Figures C&D); GB with oligodendroglial features showing strong PSMA expression in TMV & in tumor cells and gliosarcoma showing strong PSMA expression in TMV with negative expression in tumor cells respectively (IHC, original power x100).

Table 1. Clinicopathological Characteristics of the Studied Cases of HGGs and Its Correlation with PSMA Expression in TMV.

Parameter			PSMA expression in TMV								Total number of studied cases (100%)	P value
			Negative staining		Positive weak staining		Positive moderate staining		Positive strong staining			
			Number	%	Number	%	Number	%	Number	%		
Gender	Male		9	25.70	14	40.00	11	31.40	1	2.90	35	**0.579
	Female		7	20.60	12	35.30	11	32.40	4	11.80	34	
Tumor site	Cerebral	Frontal	6	40.00	5	33.30	3	20.00	1	6.70	15	*0.266
		Parietal	1	10.00	5	50.00	4	40.00	0	0.00	10	**0.598
		temporal	3	21.40	3	21.40	6	42.90	2	14.30	14	
		Occipital	1	20.00	3	60.00	1	20.00	0	0.00	5	
		more than one lobe	1	7.70	7	53.80	3	23.10	2	15.40	13	
	Deeply seated		4	40.00	2	20.00	4	40.00	0	0.00	10	
	Cerebellar		0	0.00	1	50.00	1	50.00	0	0.00	2	
Histological subtype	Anaplastic astrocytoma		4	44.40	4	44.40	1	11.10	0	0.00	9	*0.022
	Anaplastic oligodendroglioma		3	50.00	1	16.70	2	33.30	0	0.00	6	**0.163
	Anaplastic ependymoma		0	0.00	1	50.00	1	50.00	0	0.00	2	
	classic GB		1	5.00	6	30.00	9	45.00	4	20.00	20	
	GB with oligodendroglial features		0	0.00	5	62.50	2	25.00	1	12.50	8	
	Recurrent GB		4	36.40	4	36.40	3	27.30	0	0.00	11	
	Gliosarcoma		4	30.80	5	38.50	4	30.80	0	0.00	13	
Histological grade	III		7	41.20	6	35.30	4	23.50	0	0.00	17	*0.054 **0.191
	IV		9	17.30	20	38.50	18	34.60	5	9.60	52	

*P-value according to positivity and negativity; ** P-value according to composite PSMA score; Chi square test

Table 2. Clinicopathological Characteristics of the Studied Cases of HGGs and Its Correlation with PSMA Expression in TC.

Parameter			PSMA expression in TC								Total number of studied cases (100%)	P value
			Negative staining		Positive weak staining		Positive moderate staining		Positive strong staining			
			Number	%	Number	%	Number	%	Number	%		
Gender	Male		15	42.90	17	48.60	2	5.70	1	2.90	35	**0.314
	Female		15	44.10	13	38.20	6	17.60	0	0.00	34	
Tumor site	Cerebral	Frontal	7	46.70	6	40.00	1	6.70	1	6.70	15	*0.372
		Parietal	2	20.00	8	80.00	0	0.00	0	0.00	10	**0.445
		temporal	5	35.70	6	42.90	3	21.40	0	0.00	14	
		Occipital	3	60.00	1	20.00	1	20.00	0	0.00	5	
		more than one lobe	7	53.80	4	30.80	2	15.40	0	0.00	13	
	Deeply seated		6	60.00	3	30.00	1	10.00	0	0.00	10	
	Cerebellar		0	0.00	2	100.00	0	0.00	0	0.00	2	
Histological subtype	Anaplastic astrocytoma		2	22.20	6	66.70	1	11.10	0	0.00	9	*<0.001
	Anaplastic oligodendroglioma		3	50.00	2	33.30	1	16.70	0	0.00	6	**<0.001
	Anaplastic ependymoma		0	0.00	1	50.00	0	0.00	1	50.00	2	
	classic GB		2	10.00	13	65.00	5	25.00	0	0.00	20	
	GB with oligodendroglial features		5	62.50	2	25.00	1	12.50	0	0.00	8	
	Recurrent GB		7	63.60	4	36.40	0	0.00	0	0.00	11	
	Gliosarcoma		11	84.60	2	15.40	0	0.00	0	0.00	13	
Histological grade	III		5	29.40	9	52.90	2	11.80	1	5.90	17	*0.178
	IV		25	48.10	21	40.40	6	11.50	0	0.00	52	**0.207

*P-value according to positivity and negativity; ** P-value according to composite PSMA score; Chi square test

Regarding the glioma histological subtypes, PSMA expression was associated with a statistically significant relationship in both TMV and in TC and various glioma subtypes (P-value < 0.05 and <0.001 respectively). Positive PSMA immunostaining in TMV was detected in all anaplastic ependymoma cases and in near all cases of classic GB and GB with oligodendroglial features more than other subtypes, with P-value specifically for PSMA positivity/negativity in TMV statistically significant (0.022). While for Tumor cells, Positive PSMA immunostaining was detected in all anaplastic ependymoma, most anaplastic astrocytoma and classic GB cases in contrary to other variants, with P-value statistically extremely significant (< 0.001).

For PSMA expression in classic GB versus GB histologic variants "GB with oligodendroglial features, and Gliosarcoma", it was predominant in the TMV of a great proportion of classic GB and its variants (statistically insignificant, P-value > 0.05), alternatively, it decreased significantly in TC of GB with oligodendroglial features, and Gliosarcoma compared to classic GB (P-value= 0.009 and <0.001 respectively). For PSMA expression in recurrent GB, it was significantly predominant in TMV of 95% and TC of 90% of classic GB cases versus 63.5% and 36.4% of recurrent GB (P-value= 0.042 and 0.003 respectively).

Comparing PSMA IHC expression in TMV and its expression in TC, it was significantly expressed in TMV of 82.7% versus TC of 51.9% of grade IV cases. Likewise, in GB with oligodendroglial features and gliosarcoma, the majority of cases showed positive staining in their TMV [8/8 (100%), 9/13 (69.2%) respectively], and, the reverse occurs in tumor cells where the majority of cases did NOT show staining in the tumor cells for PSMA (5/8 (62.5%), 11/13 (84.6%) of cases respectively), which was statistically significant (P-value ≤ 0.05) besides the significant difference in the pattern of staining according to composite PSMA scoring (P-value ≤ 0.05).

When comparing the composite PSMA score in TMV and in tumor cells among classic GB cases, it was statistically significantly different (P-value: 0.036) as it was predominantly moderately and weakly positive (45% and 30% respectively) in TMV followed by strong staining in 20% of cases, while in tumor cells, it was predominantly weak (65% of cases) with no strong staining detected. On the other hand, in grade III and the rest of the glioma subtypes, no statistically significant difference was observed, as shown in (Table 3).

Discussion

In the present study, the mean age was 43.54 ± 15.1 years, ranging from 7 to 74 years which was near to what was reported by Barakat et al., (2016); Gabal et al., (2018) and Saffar et al., (2018), where their patients' mean age was 50, 37.23 & 46 years respectively.

As regards the gender of cases, the males and females were nearly in equal proportion with slightly higher male incidence, which was slightly lower than Barakat et al., (2016), Hewedi et al., (2018) and Pouchieu et al., (2018) who reported male incidence as 60%, 63.3 % and 57.9% of cases respectively. The male gender predominance was also reported by CBTRUS Statistical Report which stated that the incidence was higher in males for most gliomas (Ostrom et al., 2021).

PSMA expression was detected in endothelial cells of TMV as well as in TC of our high grade glioma studied cases "(53/69) (76.8%) and 39/69 (56.5%)" of cases respectively. This goes with the fact that PSMA is significantly expressed by the endothelial cells of tumor vasculature in various solid neoplasms including glial tumors especially higher-grade gliomas (Wernicke et al., 2011; Nomura et al., 2014; Matsuda et al., 2018; Saffar et al., 2018; Mahzouni and Shavakhi, 2019; Gao et al., 2021; Traub-Weidinger et al., 2021 and Holzgreve et al., 2021). For PSMA expression in TC, our results were in concordance with Nomura et al., (2014) and Holzgreve et al., (2021) who detected positive PSMA immunostaining

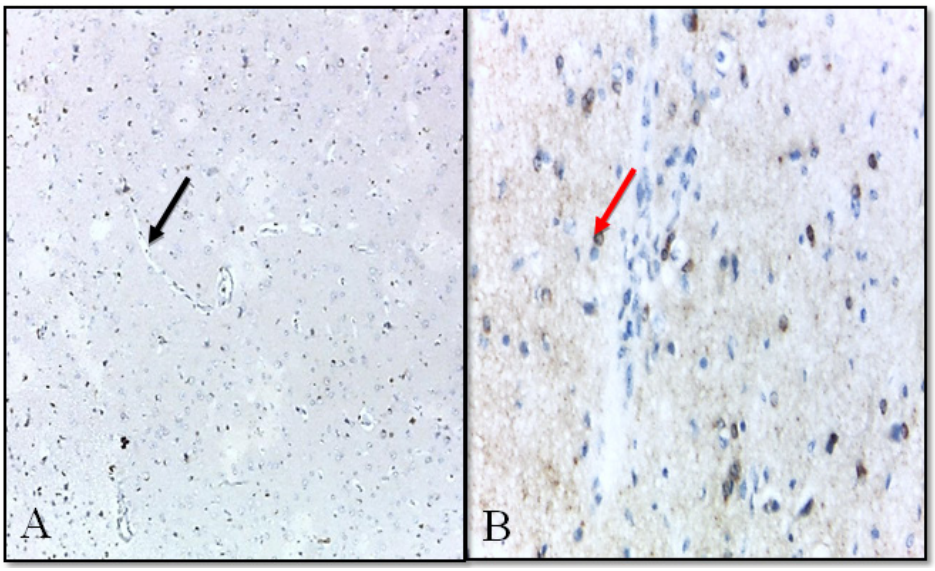

Figure 4. Normal Brain. (A) Negative normal blood vessels for PSMA (black arrows), (B) Positive some neural cells for PSMA (red arrow) (IHC, original power x100 & x400 respectively).

Table 3. Comparison between PSMA Expression in TMV and TC according to Histological Grade and Histological Subtype.

Tumor parameter		PSMA expression in TMV				PSMA expression in tumor cells				Total number of studied cases	P- value
		Positive		Negative		Positive		Negative			
		Number	%	Number	%	Number	%	Number	%	-100%	
Tumor grade	III	10	58.80	7	41.20	12	70.60	5	29.40	17	*0.473
											**0.476
	IV	43	82.70	9	17.30	27	51.90	25	48.10	52	*<0.001
											**<0.001
Tumor histological subtype	Anaplastic astrocytoma	5	55.60	4	44.40	7	77.80	2	22.20	9	*0.620
											**0.793
	Anaplastic oligodendroglioma	3	50.00	3	50.00	3	50.00	3	50.00	6	*1
											**1
	Anaplastic ependymoma	2	100.00	0	0.00	2	100.00	0	0.00	2	*…
											**1
	GB	19	95.00	1	5.00	18	90.00	2	10.00	20	*1
											**0.036
	GB with oligodendroglial features	8	100.00	0	0.00	3	37.50	5	62.50	8	*0.026
											**0.032
	Recurrent GB	7	63.60	4	36.40	4	36.40	7	63.60	11	*0.201
											**0.196
	Gliosarcoma	9	69.20	4	30.80	2	15.40	11	84.60	13	*0.005
											**0.011

*P-value according to positivity and negativity; ** P-value according to composite PSMA score; Chi square test

in TC of "grade I-II-III gliomas" and "GB between initial diagnosis and recurrence" respectively, as well as Traub-Weidinger et al., (2021) study done on 122 treatment naïve glioma patients, although little nonvascular cell staining was observed in their cases. Moreover, PSMA expression in TC was observed by Kasoha et al., (2017) "in primary breast tumors and distant metastases" and many other studies were done in different organ neoplasms as in salivary gland, lung, and liver, and certainly Prostate cancer where PSMA is overexpressed in TC more than normal and hyperplastic tissues and greater Prostatic tumor grade, disseminated cancers, and hormone-irresponsive tumors (Lütje et al., 2015 and Uijen et al., 2021).

In the current study, PSMA immunostaining was predominantly cytoplasmic. PSMA expression was not detected in normal blood vessels found in normal brain occasionally admixed with our cases, which was in agreement with Wernicke et al., (2011), Evans et al., (2016), Saffar et al., (2018), Mahzouni and Shavakhi (2019) and Gao et al., (2021), who did not find expression in the normal brain tissue. Even though in the present work, PSMA was expressed by some cells in the normal brain which coincides with Nomura et al., (2014) who identified a small subset of PSMA positive cells in normal brain tissue, while the microvasculature had no staining, and with Sacha et al., (2007) who described variable levels of cellular PSMA depending on the anatomical area of the brain.

The cerebral hemispheres were the most commonly affected site in our study representing 82.6% of cases with the frontal and temporal most commonly affected lobes (21.7% & 20.3% of all cases respectively). That was in agreement with the CBTRUS Statistical Report which declared that most gliomas were predominantly located in the supra-tentorium and only a small percentage of gliomas were found in areas of the CNS other than the brain (Ostrom et al., 2021). Furthermore, that was concordant with Mahzouni and Shavakhi (2019) study and Vallejo-Armenta et al., (2021) where the frontal lobe was the most commonly affected site (30% and 54.5% respectively), however, there was no statistically significant relationship between either PSMA expression in TMV or in TC and the site of the tumor (P-value > 0.05), which was in agreement with Mahzouni and Shavakhi (2019).

Positive immunostaining was detected in the TMV of the majority of cases (53/69) (76.8%), with grade IV more than grade III as follows; (43/52) (82.7%) and (10/17) (58.8%) respectively, Although didn't rich statistical significance; the P-value which was specifically for just *PSMA expression; positive or negative was =0.054. While according to the pattern of staining, it was predominantly weak and moderate staining while strong staining only detected in grade IV. The relationship between tumor grade and PSMA expression in TMV was statistically insignificant (P-value > 0.05) specifically for **PSMA scoring.

Our results for WHO grade IV gliomas were in alignment with but less than Wernicke et al., (2011) and Gao et al., (2021) studies; where PSMA was observed in (100%) "32/32" and "60/60" of GB cases respectively. Likewise, our results for WHO grade IV gliomas were in concordance with but higher than Mahzouni and Shavakhi (2019) report, where considerable PSMA expression level was detected in 66%. The different observations in the pattern of staining could be due to that each study might have utilized a different type of monoclonal antibody, a different IHC technique, or a different scoring system.

In the present study, PSMA immunoreactivity in TMV increases with higher grade, which coincides with what was reported by Nomura et al., (2014) study where WHO grade IV glioma (n = 5) blood vessels stained heavily for PSMA, whereas, in the lower grades II and III (n = 4 and 5 cases respectively), no blood vessels were stained. The authors hypothesized that the differential expression of PSMA in the neovasculature of different grades of gliomas may be related to the fact that GB is extremely angiogenic when compared to lower grades (Nomura et al., 2014).

Moreover, Saffar et al., (2018) demonstrated that HGGs significantly more commonly had positive results for PSMA than lower grades; where WHO grade IV glioma blood vessels stained for PSMA in 11/27 (40.7%), in contrast to, the lower grades I, II, and III [2/10 (20%), 1/26 (3.8%) and 1/9 (11.1%) respectively]. Similarly, in Matsuda et al., (2018) study, the majority of HGGs, particularly GB 40/41 (97.6%), showed positive and high PSMA expression in the tumor vascular endothelium, significantly higher than in grades I, II, and III gliomas, 3/4 (75%), 1/7 (14.3%) and 10/15 (66.7%), respectively, with predominant high PSMA expression in higher grades gliomas. Furthermore, in the entire cohort study done by Traub-Weidinger et al., (2021), vascular PSMA expression was observed in 26 out of 122 glioma cases (21% of cases), whereas the vast majority of GB 21/26 (81%) showed significantly higher vascular PSMA expression, 4/40 (10%) of glioma grade III and 1/56 (2%) grade I-II had vessel staining. According to the glioma subtype, Positive PSMA immunostaining in TMV was detected much more in anaplastic ependymoma in comparison to anaplastic astrocytoma and anaplastic oligodendroglioma (100%, 55.6%, and 50% respectively), while in grade IV, Positive PSMA immunostaining was detected in near all cases of classic GB and GB with oligodendroglial features more than other variants, with predominant weak and moderate staining in all positive cases, The relation between the glioma subtype and PSMA positivity/negativity in TMV was statistically significant (P-value < 0.05).

Traub-Weidinger et al., (2021) studied 122 naive cases, the cases were primarily evaluated according to the 2007 WHO classification as our study. In the subgroup of WHO 2007 classified gliomas, our results for grade III were higher; since only 1/16 (6%) of anaplastic astrocytoma, 3/24 (12.5%) of anaplastic oligodendroglioma and anaplastic oligoastrocytoma showed positive PSMA staining in the blood vessels.

As regards PSMA expression in TMV in GB histologic variants versus classic GB, PSMA expression was predominant in the TMV of a great proportion of cases (95% of classic GB, 100% of GB with oligodendroglial features, and 69.2% of Gliosarcoma), which was statistically insignificant (P-value > 0.05), these findings were not tested by other comparative studies.

Concerning the relationship between PSMA expression in TMV and the recurrence of GB, PSMA expression was significantly predominant in the TMV of 95% of classic GB cases (presented with primary lesion) versus 63.5% of recurrent GB in our study, which was similar to but less than Holzgreve et al., (2021) and Vallejo-Armenta et al., (2021) studies, where PSMA was expressed in the great majority of recurrent cases, thus PSMA expression might present a hopeful target for theranostic approaches.

Concerning PSMA expression in glial TC, Positive immunostaining was detected in nearly half of cases 39/69 (56.5%), with grade III higher than grade IV as follows; 12/17 (70.6%) and 27/52 (51.9%) respectively, with predominant weak staining followed by moderate and strong staining. However, the relationship between tumor grade and PSMA expression in TC was statistically insignificant.

Our results were in concordance but much higher than Nomura et al., (2014) where grade III (5 cases) exhibited some staining of TC with no staining detected in grade IV (5 cases). Likewise, Traub-Weidinger et al., (2021) reported that the quantity of non-vascular PSMA positivity in the glioma tissue samples studied did not change substantially between higher and lower grade gliomas.

In Kasoha et al., (2017) study done on breast cancer, PSMA expression in TC appeared in a great proportion of cases "72%" (49/68) and ranged between weak and moderate similar to our results, in spite of being studied in breast cancer cases, yet higher grade 3 showed 100% PSMA positive immunoreactivity (15/15) which is more than lower grades 1 and 2 "64.1%" (34/53) in contrary to our results where PSMA expression in TC in higher grade IV is less than lower grade III.

As regards the expression of PSMA in TC according to glioma subtype, Positive PSMA immunostaining was detected much more in anaplastic ependymoma and anaplastic astrocytoma in comparison to anaplastic oligodendroglioma (100%, 77.8%, and 50% respectively), while in grade IV, Positive PSMA immunostaining was detected mainly in the majority of classic GB cases (90%) in contrary to other variants, with predominant weak staining in all positive cases, The relation between glioma subtype and PSMA expression in TC was statistically extremely significant (P-value < 0.001).

Similarly, for PSMA expression in classic GB versus GB histologic variants, PSMA expression in TC was significantly predominant in 90% of classic GB cases versus 37.5% of GB with oligodendroglial features and 15.4% of Gliosarcoma (P-value= 0.009 and <0.001 respectively). In fact, these findings were not thoroughly evaluated by other comparative studies; Since little information exists about PSMA expression in TC of brain tumors.

Concerning the relationship between PSMA expression in TC and the recurrence of GB, PSMA expression decreased significantly in recurrent GB (36.4%) versus classic GB cases (90%) (presented with primary lesion) in the current study in contrast to TMV in our cases, this was in agreement with Vallejo-Armenta et al., (2021) who declared that PSMA was strongly expressed by the endothelium of GB and recurrent GB with an overall minimal PSMA expression in TC in all cases. Nevertheless, in Holzgreve et al., (2021) study done on GB (to compare between PSMA expression at first and second surgery), PSMA is expressed in the majority of cases in TC (14/16) "87.5%"; in the overall group of GB, PSMA staining within the tumor increases somewhat between first diagnosis and recurrence, this could be explained due to that the recurrent GB cases, in our and Vallejo-Armenta et al., (2021) studies, were not examined at their initial diagnosis for PSMA and they might be initially negative.

When Comparing PSMA expression in TMV and PSMA expression in TC in the studied cases, in grade III, Positive PSMA expression was detected in TC in (12/17) (70.6%) more than in TMV (10\17) (58.8%) of cases, however, it was statistically insignificant (P-value > 0.05). While In grade IV, Positive PSMA expression was detected mainly in TMV in (43\52) (82.7%) more than TC in (27/52) (51.9%) of cases, which was statistically extremely significant (P-value < 0.001).

According to the glioma subtype, in GB with oligodendroglial features and in gliosarcoma, the majority of cases showed positive immunostaining in their TMV, and, the reverse occurs in TC where the majority of cases did NOT show staining in the TC for PSMA, which was statistically significantly different in addition to a significant difference in the pattern of staining according to composite PSMA scoring (P-value ≤ 0.05).

In the rest of the glioma subtypes, there was no statistically significant difference between PSMA expression in TMV and PSMA expression in TC (either positivity/ negativity or the pattern of staining according to composite PSMA scoring) with exception in classic GB, the composite PSMA score in TMV and in TC among classic GB cases was statistically significantly different, P-value: 0.036. However, as previously mentioned, these observations were not thoroughly evaluated by other comparative studies.

Evidently, from this study's results, PSMA was expressed in a significant proportion of TMV and TC of HGGs, Thus, PSMA might present a promising target for imaging and subsequent treatment using agents that are capable of inhibiting PSMA's function or delivering chemotherapeutics or radiation agents via PSMA-mediated delivery.

Author Contribution Statement

All authors contributed efficiently to the research and approved the manuscript. MGS and MSA shared in study design and sample collection. MGS and SMF shared in writing the manuscript. All authors shared in results analysis, interpretation, revising, and approving the final version of the manuscript. .

Acknowledgements

Approval

This study was approved by the research committee of pathology department, faculty of medicine, Cairo University, and research ethics committee (REC) committee.

Ethical Declaration

This study obtained the approval of the Kasr Alainy REC that conducts according to ICH GCP standards and appropriate local and institutional regulations and guiding principles that govern REC operation (N-205-2020).

Availability of Data

Data is available upon request according to the institutional regulations and with official permission.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Barakat MK, Belal AM, Fadel SH, et al (2016). Outcome of high grade gliomas in limited resource country (10 years' experience in Alexandria University Oncology Center 2003-2012). *J Brain Tumors Neurooncol*, **1**, 2.

Brem S, Cotran R, Folkman J (1972). Tumor angiogenesis: a quantitative method for histologic grading. *J Natl Cancer I*, **48**, 347-56.

Bush NA, Chang SM, Berger MS (2017). Current and future strategies for treatment of glioma. *Neurosurg Rev*, **40**, 1-14.

Chan YH (2003). Biostatistics 103: qualitative data-tests of independence. *Singap Med J*, **44**, 498-503.

Chang SS, Reuter VE, Heston WD, et al (1999). Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. *Cancer Res*, **59**, 3192-8.

Evans JC, Malhotra M, Cryan JF, et al (2016). The therapeutic and diagnostic potential of the prostate-specific membrane antigen/glutamate carboxypeptidase II (PSMA/GCPII) in cancer and neurological disease. *Br J Pharmacol*, **173**, 3041-79.

Gabal SM, Elsheikh SA, Abdelmaged MS, Sabry RM (2018). Expression of Extracellular Matrix Metalloproteinase Inducer (CD147) in Astrocytomas: A Histopathological and Immunohistochemical Study. *J Clin Diagn Res*, **12**, 9-13.

Gao Y, Zheng H, Li L, et al (2021). Prostate-Specific Membrane Antigen (PSMA) Promotes Angiogenesis of GlioblastomaThrough Interacting with ITGB4 and Regulating NF-κB Signaling Pathway. *Front Cell Dev Biol*, **9**, 462.

Grant CL, Caromile LA, Durrani K, et al. (2012). Prostate specific membrane antigen (PSMA) regulates angiogenesis independently of VEGF during ocular neovascularization. *PLoS One*, **7**, e41285.

Haffner MC, Laimer J, Chaux A, et al (2012). High expression of prostate-specific membrane antigen in the tumor-associated neo-vasculature is associated with worse prognosis in squamous cell carcinoma of the oral cavity. *Modern Pathol*, **25**, 1079- 85.

Hewedi I, Ibrahim R, Elserry T, et al (2018). Frequency of primary central nervous system tumors in a tertiary hospital, Cairo, Egypt. *J Commun Health*, **5**, 140-6.

Holzgreve A, Biczok A, Ruf VC, et al (2021). PSMA expression in glioblastoma as a basis for theranostic approaches: a retrospective, correlational panel study including immunohistochemistry, clinical parameters and PET imaging. *Front Oncol*, **11**, 861.

Jayson GC, Kerbel R, Ellis LM, et al (2016). Antiangiogenic therapy in oncology: current status and future directions. *Lancet*, **388**, 518-29.

Juratli TA, Schackert G, Krex D (2013). Current status of local therapy in malignant gliomas—a clinical review of three selected approaches. *Pharmacol Ther*, **139**, 341- 58.

Kasoha M, Unger C, Solomayer EF, et al (2017). Prostate-specific membrane antigen (PSMA) expression in breast cancer and its metastases. *Clin Exp Metastasis*, **34**, 479-40.

Louis DN, Ohgaki H, Wiestler OD, et al (2007). The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*, **114**, 97-109.

Lütje S, Heskamp S, Cornelissen AS, et al (2015). PSMA ligands for radionuclide imaging and therapy of prostate cancer: clinical status. *Theranostics*, **5**, 1388.

Mahzouni P, Shavakhi M (2019). Prostate-specific membrane antigen expression in neovasculature of glioblastoma multiforme. *Adv Biomed Res*, **8**, 18.

Matsuda M, Ishikawa E, Yamamoto T, et al (2018). Potential use of prostate-specific membrane antigen (PSMA) for detecting the tumor neovasculature of brain tumors by PET imaging with 89Zr-Df-IAB2M anti-PSMA minibody. *J Neurooncol*, **138**, 581-9.

Mhawech-Fauceglia P, Zhang S, Terracciano L, et al (2007). Prostate specific membrane antigen (PSMA) protein expression in normal and neoplastic tissues and its sensitivity and specificity in prostate adenocarcinoma: an immunohistochemical study using mutiple tumour tissue microarray technique. *Histopathology*, **50**, 472-83.

Moore M, Panjwani S, Mathew R, et al (2017). Well-differentiated thyroid cancer neovasculature expresses prostate-specific membrane antigen - a possible novel therapeutic target. *Endocr Pathol*, **28**, 339-44.

Nomura N, Pastorino S, Jiang P, et al., (2014). Prostate specific membrane antigen (PSMA) expression in primary gliomas and breast cancer brain metastases. *Cancer Cell Int*, **14**, 1-9.

Ostrom Q, Cioffi G, Waite K, et al (2021). CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2014–2018. *Neurooncology*, **23**, 1-105.

Pouchieu C, Raherison C, Piel C, et al (2018). Allergic conditions and risk of glioma and meningioma in the CERENAT case-control study. *J Neurooncol*, **138**, 271-81.

Reifenberger G, Wirsching HG, Knobbe-Thomsen CB, et al. (2017). Advances in the molecular genetics of gliomas - implications for classification and therapy. *Nat Rev Clin Oncol*, **14**, 434-452.

Ruigrok EA, van Weerden WM, Nonnekens J, et al (2019). The future of PSMA-targeted radionuclide therapy: an overview of recent preclinical research. *Pharmaceutics*, **11**, 560.

Šácha P, Zámečník J, Bařinka C, et al (2007). Expression of glutamate carboxypeptidase II in human brain. *Neurosciences*, **144**, 1361-72.

Saffar H, Noohi M, Tavangar SM, et al (2018). Expression of prostate-specific membrane antigen (PSMA) in brain glioma and its correlation with tumor grade. *Iran J Pathol*, **13**, 45.

Tagawa ST, Osborne JR, Vallabhajosula S, et al (2013). Prostate-Specific Membrane Antigen-Based Therapeutics. In Prostate Cancer: A Comprehensive Perspective, Springer, London, pp 459-66.

Thorpe PE (2004). Vascular targeting agents as cancer therapeutics. *Clin Cancer Res*, **10**, 415-27.

Traub-Weidinger T, Poetsch N, Woehrer A, et al (2021). PSMA Expression in 122 Treatment-Naive Glioma Patients Related to Tumor Metabolism in 11C-Methionine PET and Survival. *J Pers Med*, **11**, 624.

Tsui P, Rubenstein M, Guinan P (2005). Correlation between PSMA and VEGF expression as markers for LNCaP tumor angiogenesis. *J Biomed Biotechnol*, **2005**, 287-90.

Uijen MJ, Derks YH, Merkx RI, et al (2021). PSMA radioligand therapy for solid tumors other than prosta te cancer:

Background, opportunities, challenges, and first clinical reports. *Eur J Nucl Med Mol I*, **48**, 4350-68.
Vallejo-Armenta P, Soto-Andonaegui J, Villanueva-Pérez RM, et al (2021). [99mTc] Tc-iPSMA SPECT brain imaging as a potential specific diagnosis of metastatic brain tumors and high-grade gliomas. *Nucl Med Biol*, **96**, 1-8.
Wernicke AG, Edgar M A, Lavi E, et al (2011). Prostate-specific membrane antigen as a potential novel vascular target for treatment of glioblastoma multiforme. *Arch Pathol Lab Med*, **135**, 1486-9.
Wernicke AG, Varma S, Greenwood EA, et al (2014). Prostate specific membrane antigen expression in tumor associated vasculature of breast cancers. *APMIS*, **122**, 482-9.
Wick W, Gorlia T, Bendszus M, et al (2017). Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med*, **377**, 1954-63.
Zalata KR, El-Tantawy DA, Abdel-Aziz A, et al (2011). Frequency of central nervous system tumors in delta region, Egypt. *Indian J Pathol Microbiol*, **54**, 299.