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Article

Immunohistochemical Study of c-erbB2/HER2 Tumor Marker in Primary Malignant Brain Tumors

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Abstract: *Background and objectives:* Primary brain tumors include any tumors arising in the brain whose prognosis is poor due to their histologic characteristics. The aim of this research was to evaluate the frequency of HER2 tumor marker in primary malignant brain tumors. *Materials and Methods:* This descriptive study was conducted on the samples admitted to the pathology laboratory with diagnosis of primary brain tumor during 2008-2015. *Results:* From among 107 patients (61.7% males and the rest females) with mean age of 40.4 years, the highest frequency of tumor location was in supratentorial region of the brain (including lobes and ventricles) (63.85% cases). High-grade astrocytoma had the highest prevalence at diagnosis (43.9%), followed by low-grade astrocytoma (37.4%). As for HER2 score, 42.1% of patients were HER2-positive (scores 2 & 3). On the other hand, 5.6% of patients were HER2-negative (-), 40.2% were positive (+), and 54.2% were positive (++). The patients with high-grade astrocytoma had older age ($P<0.001$), higher HER2 positivity ($P=0.024$) and percentage ($P<0.001$) compared to the patients with low-grade astrocytoma. *Conclusions:* HER2 expression is dependent on the type of brain tumors. High expression of HER2 in high-grade astrocytoma may be useful for therapeutic purposes. The future research is needed to confirm these results with a large number of patients in different areas.

Keywords: Primary brain tumor; HER2; immunohistochemistry

1. Introduction

Primary brain tumors include any tumors arising in the brain that can start from brain cells, meninges, nerves, or glands [1]. Their prognosis is poor due to their histologic characteristics; however, some benign tumors are located in inoperable areas [2]. In the studies reported in the U.S. and Europe, the incidence rate of brain neoplasms varies from 17.6 to 22.0 per 100000 persons [3]. Around 18,500 new cases of primary malignant brain tumors are diagnosed each year in the USA [4]. A systematic review in Iran from 2000 to 2010 reported primary brain tumors had an overall incidence of 2.74 per 100,000 persons, the most common histopathologies of which included meningioma, astrocytoma, glioblastoma multiforme (GBM), and ependymoma [5]. Astrocytic tumors are primary brain tumors that may progress to GBM, a highly malignant neoplasm of the central nervous system (CNS) [6]. Medulloblastoma is the most common malignant brain tumor in children [7,8], accounting for around 20% of all primary tumors of the CNS, with a peak incidence at ages 5-7 years [8]. Recent studies have suggested that aberrations of the human epidermal growth factor receptor 2 (HER2) or c-erbB-2 may be involved in astrocytic brain tumors [9-11].

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HER2, a 185-kD protein, is an important prognostic indicator/target for metastatic breast carcinoma therapy [12]. The HER-2/neu oncogene is located on chromosome 17q21, encodes for a 185 kd transmembrane glycoprotein with intracellular tyrosine kinase activity. HER2 overexpression is observed in 20% to 40% of breast cancers and other solid tumors and is generally correlated with a poor prognosis [13,14]. Trastuzumab (Herceptin) is the standard care for HER2-positive tumors, especially in some metastatic/advanced solid tumors (breast and gastroesophageal cancers) [15], approved by the FDA for breast cancer therapy [16]. The studies conducted in different areas on various ethnicities/races have indicated that HER2 can express in primary brain tumors, whose expression depends on tumor location and tumor type [7,8,12,17-20]. Therefore, for the first time we aimed to study the prevalence of HER2 marker in different types of primary malignant brain tumors in West of Iran and Kurdish race.

2. Materials and Methods

This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran. This descriptive study was performed on the 107 samples diagnosed with primary malignant brain tumor and admitted to the pathology laboratory of Imam Reza Hospital during 2008-2015.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections from each previously diagnosed primary brain tumor (according to WHO classification) were cut into 4 micron-thick sections and mounted on glass slides. These sections were stained by hematoxylin and eosin staining. The initial diagnosis was confirmed by the pathologist. Then, new sections were provided, and immunohistochemistry (IHC) staining was done. Primary antihuman antibody against c-erbB-2 Oncoprotein (DAKO diagnostics, Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein, Code A0485) was applied by IHC method according to the manufacturer's brochure. Only staining of the cell membrane was considered specific for c-erbB-2 oncoprotein. Positive control samples were received from strong c-erbB-2 stained breast carcinomas and negative ones from normal breast tissue. Brown color in tumor cell cytoplasmic membrane was considered c-erbB-2-positive. The percentage of stained cells and intensity of staining were graded from 0 to 3+, as follows: No staining (0), low intensity and incomplete membrane staining in less than 10% of cells (1+), low intensity and complete membrane staining in more than 10% of cells (2+), and high intensity and complete membrane staining in more than 10% of cells (3+). According to the study of Mineo et al.[18], cell staining ≤10% was considered negative (–), between 11–50% (+), and ≥51% positive (++). On the other hand, tumors with scores 0 and 1+ were considered negative, while those with scores 2+ and 3+ were considered positive [21]. **Figure 1** shows IHC staining pattern of 0 to 3+ in pilocytic and anaplastic astrocytomas.

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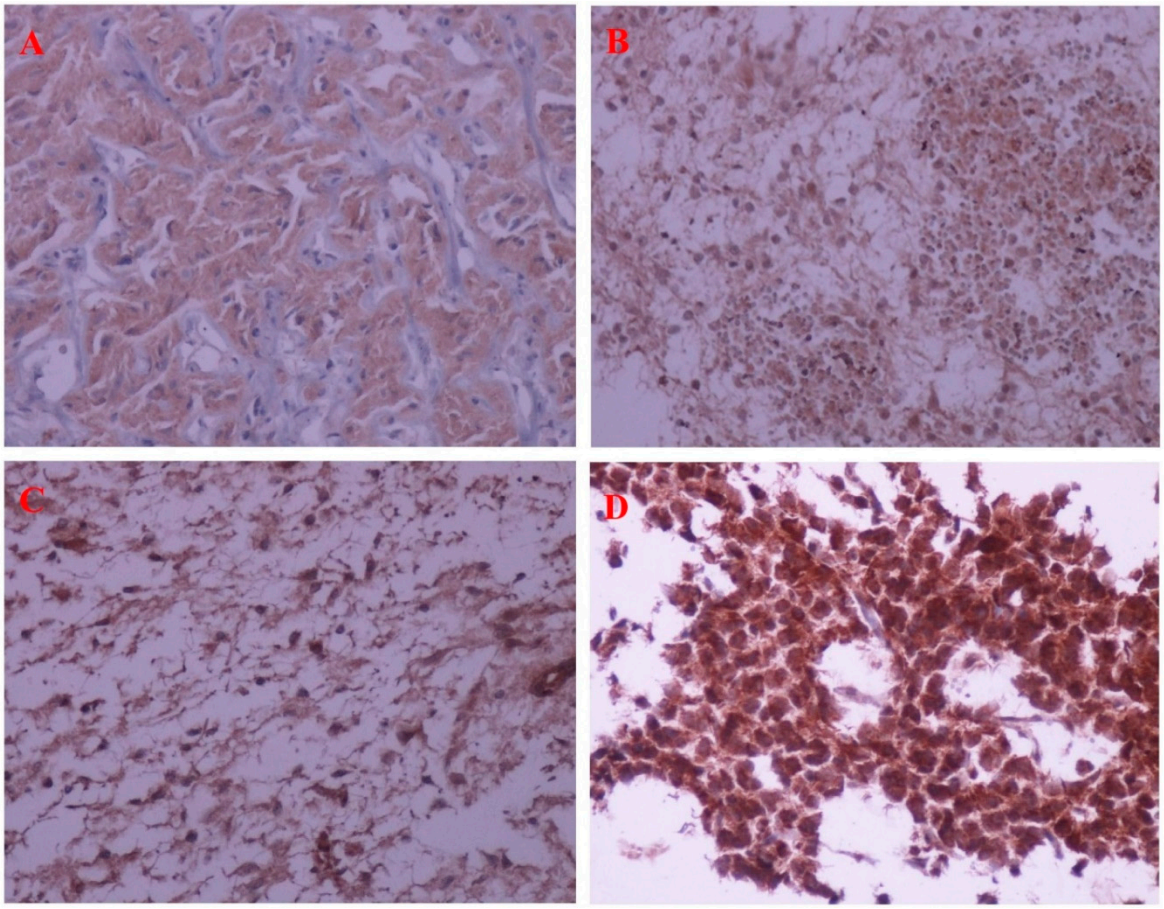

Figure 1. Immunohistochemistry staining of HER2, x200 magnification: A) Pilocytic astrocytoma (score = 0), B) Pilocytic astrocytoma (score = 1), C) Pilocytic astrocytoma (score = 2), and D) Anaplastic astrocytoma (score = 3).

Statistical analysis

The data were analyzed by SPSS software (version 16). The mean and standard deviation were calculated for age; whereas, number of patients and percentage were considered for the other data. Chi-square test was used for comparison of age between the variables and t-test for others between the variables.

Limitations

1) Losing the information of some patients. 2) Unavailability of some paraffin blocks. 3) Low number of cases in the Hospital. 4) Lack of fluorescence in situ hybridization (FISH) for HER2 (2+).

3. Results

Out of 107 patients with primary malignant brain tumors with mean age at diagnosis of 40.4 years (range, 1-88 years), 61.7% of patients were male. Reporting tumor location in 85 patients, 74.1% tumors occurred in supratentorial region. The patients had different complaints that are shown in **Table 1**. High-grade astrocytoma had the highest prevalence at diagnosis (43.9%), followed by Low-grade astrocytoma (37.4%), primitive neuroectodermal tumor small round cell tumor lymphoma medulloblastoma (15.9%), mixed oligoastrocytoma (0.9%), hemangioblastoma (0.9%), and choroid plexus papilloma (0.9%). Checking HER2 scores, 42.1% of patients were HER2-positive (scores 2 & 3). In addition, based on staining percentage of HER2, 5.6% of patients were HER2-negative (-), 40.2% were positive (+), and 54.2% were positive (++).

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Table 1. Baseline characteristics of the patients with primary brain tumors (n=107).

Variable	N (%)	Variable	N (%)
		HER2 score	
Age, year		0	6 (5.6)
Mean ± SD	40.4 ± 22.35	1	56 (52.3)
Range	1-88	2	36 (33.6)
		3	9 (8.4)
Sex		**HER2 status**	
Male	66 (61.7)	Negative	62 (57.9)
Female	41 (38.3)	Positive	45 (42.1)
Tumor location		**HER2**, %	
Sellar	2 (2.4)	Mean ± SD	55.31 ± 28.15
Supratentorial	63 (74.1)	Range	0-100
Brain stem	3 (3.5)		
Cerebellum	17 (20)		
NA	22		
Complaints			
Lateralized	18 (30)	**HER2**, %	6 (5.6)
Generalized	22 (36.7)	≤10 (-)	43 (40.2)
Mixed	20 (33.3)	11-50 (+)	58 (54.2)
NA	47	≥51 (++)	
Type of tumor			
Low-grade astrocytoma	40 (37.4)		
High-grade astrocytoma	47 (43.9)		
Mixed oligoastrocytoma	1 (0.9)		
PNET-SRCTLM	17 (15.9)		
Hemangioblastoma	1 (0.9)		
Choroid plexus papilloma	1 (0.9)		

Abbreviations: PNET-SRCTLM, primitive neuroectodermal tumor small round cell tumor lymphoma medulloblastoma; NA; Not available.

Table 2 is shown the correlation between some variables and HER2 status. There was no significant difference in age, sex, tumor location, and type of tumor between HER2-positive and HER2-negative patients.

Table 2. The correlation between variables and HER2 status in the brain tumor patients (n=107).

Variable	HER2-positive	HER2-negative	P-value
Age, year			0.582
Mean ± SD	41.44 ± 22.89	39.02 ± 22.09	
Sex			0.458
Male	27 (60%)	39 (62.9%)	
Female	18 (40%)	23 (27.1%)	
Tumor location (n=85)			
Sellar	1 (2.6%)	1 (2.2%)	
Supratentorial	30 (76.9%)	33 (71.7%)	0.934
Brain stem	1 (2.6%)	2 (4.3%)	
Cerebellum	7 (17.9%)	10 (21.7%)	
Type of tumor			
Low-grade astrocytoma	12 (26.7)	28 (45.2%)	
High-grade astrocytoma	25 (55.6%)	22 (35.5%)	
Mixed oligoastrocytoma	0 (0%)	1 (1.6%)	0.179
PNET- SRCTLM	7 (15.6%)	10 (16.1%)	
Hemangioblastoma	0 (0%)	1 (1.6%)	
Choroid plexus papilloma	1 (2.2%)	0 (0%)	

Abbreviation: PNET-SRCTLM, primitive neuroectodermal tumor small round cell tumor lymphoma medulloblastoma.

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The correlation between some variables and HER2 status showed no significant difference in the variables (age, sex, tumor location, and type of tumor) between HER2 (-), HER2 (+) positive, and HER2 (++) positive samples (**Table 3**).

Table 3. Correlation between study variables and HER2 status in the brain tumor patients (n=107).

Variable	HER2 (-)	HER2 (+)	HER2 (++)	P-value
Age, year				
Mean ± SD	36.3 ± 26.6	38.7 ± 19.8	41.4 ± 24.3	0.767
Sex				
Male	2 (33.3%)	30 (69.8%)	34 (58.6%)	0.177
Female	4 (66.7%)	13 (30.2%)	24 (41.4%)	
Tumor location (n=85)				
Sellar	0 (0%)	0 (0%)	2 (4.4%)	
Supratentorial	3 (60%)	26 (74.3%)	34 (75.6%)	0.698
Brain stem	0 (0%)	1 (2.9%)	2 (4.4%)	
Cerebellum	2 (40%)	8 (22.9%)	7 (15.6%)	
Type of tumor				
Low-grade astrocytoma	4 (66.7%)	22 (51.2%)	14 (24.1%)	
High-grade astrocytoma	1 (16.7%)	14 (32.6%)	32 (55.2%)	
Mixed oligoastrocytoma	0 (0%)	0 (0%)	1 (1.7%)	0.193
PNET- SRCTLM	1 (16.7%)	6 (14%)	10 (17.2%)	
Hemangioblastoma	0 (0%)	1 (2.3%)	0 (0%)	
Choroid plexus papilloma	0 (0%)	0 (0%)	1 (1.7%)	

Abbreviation: PNET-SRCTLM, primitive neuroectodermal tumor small round cell tumor lymphoma medulloblastoma.

Figure 2 is shown the subtypes and tumor locations of primary brain tumors more specifically. Low-grade astrocytoma II/IV (33 patients) and GBM grade IV/IV (30 patients) had the highest prevalence. In addition, parietal lobe, frontal lobe, and cerebellum with 21, 18, and 17 patients, respectively, had the highest prevalence.

Comparing the correlation between some variables among low- and high-grade astrocytomas, there was a significant difference between the two astrocytomas in the variables of age, tumor location, HER2 statues, and percentages (**Table 4**). The patients with high-grade astrocytoma had older age (P<0.001), higher HER2 positivity (P=0.024), and higher HER2 percentage (P<0.001) compared to the patients with low-grade astrocytoma. In addition, all high-grade astrocytoma tumors were in supratentorial region compared with 75% of low-grade astrocytoma tumors in this location (P=0.012).

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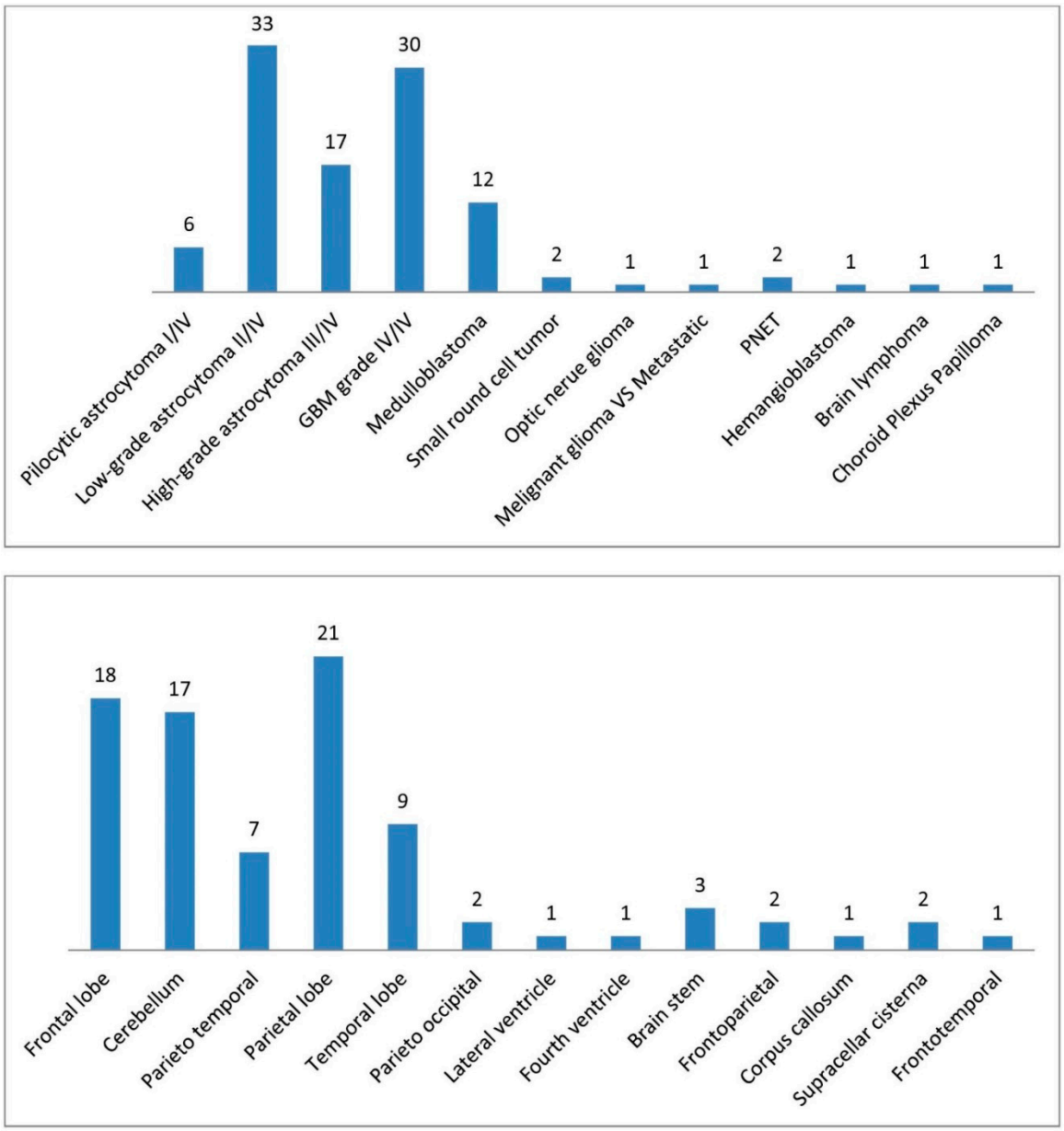

Figure 2. Prevalence of the patients with primary brain tumors, (A) subtypes and (B) tumor locations.

Table 4. Correlation between study variables and astrocytic tumors in the brain tumor patients (n=87).

Variable	Low-grade astrocytoma	High-grade astrocytoma	P-value
Age, year			
Mean ± SD	35.6 ± 20.6	50.8 ± 16.2	**<0.001**
Sex			
Male	24 (60%)	31 (66%)	0.362
Female	16 (40%)	16 (34%)	
Tumor location (n=67)			
Sellar	2 (7.1%)	0 (0%)	
Supratentorial	21 (75%)	39 (100%)	**0.012**
Brain stem	1 (3.6%)	0 (0%)	
Cerebellum	4 (14.3%)	0 (0%)	
HER2 status			
Negative	28 (70%)	22 (46.8%)	**0.024**

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Positive	12 (30%)	25 (53.2%)	
HER2, %			
Mean ± SD	42.4 ± 28.1	65.4 ± 24.6	**<0.001**
Range	0-100	5-100	
HER2, %			
≤10 (-)	4 (10%)	1 (2.1%)	**0.006**
11-50 (+)	22 (55%)	14 (29.8%)	
≥51 (++)	14 (35%)	32 (68.1%)	

4. Discussion

The present study analyzed HER2 expression in primary brain tumors. The results showed that 42.1% of patients were HER2-positive. In addition, 40.2% and 54.2% of tumors in the patients were (+) and (++) positive, respectively. In comparison of low- versus high-grade astrocytomas, Her2 positivity was significantly higher in high-grade than low-grade tumors.

One study [21] investigated 72 patients with low- and high- grade astrocytomas (56.9% GBM, 13.9% diffuse astrocytoma, and 20.8% anaplastic astrocytoma) for HER2 overexpression, whose results showed 23.6% of patients were HER2-positive. There was no HER2 positivity in diffuse astrocytoma and pilocytic astrocytoma specimens, and overexpression was observed only in GBM subtype. In Reszeć's study [6] on sixty-five patients with astrocytic tumors, including 17 diffuse astrocytoma, 23 anaplastic astrocytoma, and 25 GBM, HER2 expression was observed in 88.3%, 88%, and 82.6% of diffuse astrocytoma, anaplastic astrocytoma, and GBM, respectively. In GBM, 11 samples (47.8%) were (+) positive and only 8 (34.8%) tumors were (++) positive. In 11/22 medulloblastoma tumors, 10 to 50% of the tumor cells showed HER2 and HER4 positivity, which were detectable only in high-grade glial tumors [22]. Torp et al. [10] found HER2 positivity in 9 of 21 GBM tumors (43%).

A study [23] included 44 GBM patients (61.4% males) with mean age of 79 years. All tumors were negative for HER-2/neu protein by IHC and for HER-2/neu gene amplification by FISH. Happasalo et al.[9] confirmed this result with astrocytic neoplasms. Meurer et al.[17] analyzed 40 medulloblastoma tumors and found that HER2 was positive in 23 patients (57.5%). In a retrospective study [18] on 57 patients with GBM and 16 patients with grade III gliomas, all GBM tumors expressed HER2 (2+ and 3+) highly and all secondary GBM tumors expressed HER2 with low intensity (0 and 1+). A series of 70 cases with childhood medulloblastoma were analyzed for HER2 expression by IHC, sixty of which (85.7%) were found to be positive in IHC analysis. Ahmed et al.[7] showed 40% HER2 expression in medulloblastomas. Out of 149 GBM cases (54.4% males and mean age of 64 years); HER2 overexpression was detected in 23 patients (15.4%) [12]. Potti et al.[24] checked 347 adult patients (55.6% males and mean age of 53 years) with primary malignant brain tumors. It was found that 10.4% of the archival pathologic samples showed presence of HER-2/neu overexpression by IHC.

Gulati et al.[19] investigated HER2 expression in 31 cases with anaplastic astrocytomas with three monoclonal antibodies, including CB11, 3B5, and 5A2. HER2 positivity was observed in 45%, 100%, and 52% of cases, respectively. This discrepancy in results may be due to using different monoclonal antibodies and different interpretations of the positive samples. Mineo et al.[18] showed survival time was significantly longer when HER2 expression was low in the patients with medulloblastoma tumors. Koka et al. [12], after adjusting for age, performance status, smoking history, and treatment of GBM patients, revealed that HER2 overexpression significantly increased the odds of early mortality (the median survival of patients for HER2 overexpression was 4 months compared to 8 months for lack of overexpression), which were confirmed by Potti et al.[24] Therefore, the results showed that HER2 overexpression may be a poor prognostic marker in patients with GBM [12] and astrocytic tumors of the brain [22]. The studies suggest that HER2 expression may be involved in the development and progression of astrocytic brain tumors and may be potentially significant owing to the role of Herceptin therapy in these tumors [8,25,26].

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5. Conclusions

Considering the high expression of HER2 in most brain tumors, HER2 overexpression may be a poor prognostic marker in patients with primary brain tumors. But the results showed HER2 expression is dependent on the type of brain tumors. High expression in high-grade astrocytoma may be useful for therapeutic purposes. Future studies are recommended to be conducted on a large number of patients in different areas to confirm these results.

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Conflicts of Interest: None to declare.

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