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Describing the Expression Profiles of Glutathione S-Transferase Mu and Tumor Protein 53 in Brain Tumor Tissue

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ABSTRACT

Objective: This study aims to explore the expression profiles of the glutathione S-transferase-Mu (GST-M) isozyme and tumor protein 53 (p53) in both healthy and tumorous brain tissues. The findings are compared with clinical features and lifestyle factors to identify potential associations or correlations.

Materials and Methods: We retrospectively analyzed the medical records of 149 patients diagnosed with primary or metastatic intracranial tumors. The expression levels of GST-M and p53 proteins were assessed in healthy and tumorous brain tissues using immunohistochemical staining. We also evaluated the associated clinical features and lifestyle factors.

Results: There was a significant difference in the expression levels of GST-M between tumorous and healthy brain tissues, with tumor tissues showing higher expression ($p<0.0001$). Conversely, robust p53 expression was absent in both normal (97.3%) and tumor (78.5%) tissues. Nevertheless, a significantly higher prevalence of samples with p53 expression was found in the tumor group ($p<0.0001$). No associations were found between expression levels and clinical features or lifestyle risk factors. Furthermore, GST-M and p53 expression did not impact postoperative survival rates.

Conclusion: The findings indicate an elevated expression of GST-M in brain tumor tissues, suggesting a potential role for GST-M in brain tumorigenesis.

Keywords: Brain cancer, immunohistochemistry, glutathione S-transferase, tumor protein 53, lifestyle.

INTRODUCTION

Tumors originating in the central nervous system (CNS), which includes the brain and spinal cord, pose a significant health problem due to their interference with essential bodily functions controlled by the CNS. These tumors can be classified as secondary (originating from metastasis) or primary (developing within the central nervous system itself). Common types of CNS tumors include astrocytomas, oligodendrogliomas, ependymomas, medulloblastomas, and meningiomas.[1]

Brain and central nervous system tumors are particularly lethal, characterized by high morbidity and mortality rates. In 2016, there were 330,000 global cases, translating to an age-standardized rate of 4.63 per 100,000 person-years.[2] Recent meta-analyses reveal that there are approximately 3.6 primary CNS tumors per 100,000 individuals annually.[3] In 2019, there were 347,992 new cases and 246,253 deaths worldwide, with an age-standardized mortality rate of 3.05 per 100,000 people.[4] CNS tumors continue to be a serious health challenge in Türkiye, albeit with relatively decreased incidence rates. In 2020, 6,102 cases in Türkiye resulted in 5,070 fatalities, with a five-year prevalence rate of 20.2 per 100,000 population.[5]

The etiology of CNS tumors is multifactorial, involving genetic, environmental, and lifestyle elements that influence both the development of cancer and its prognosis. Prognosis in brain cancer is challenging due to limited chemotherapy (CT) options. Clinical factors such as tumor type, location, size, and grade are crucial in determining patient outcomes.[6] Although lifestyle factors like body mass index, alcohol consumption, and smoking are linked to cancer prognosis broadly, their specific effects on brain tumors are not yet clearly understood.

Genetic factors impact CT responses and overall prognosis in CNS tumors. Polymorphisms in drug-metabolizing enzymes influence drug metabolism, while detoxification mechanisms, antioxidants, and enzyme expressions, particularly those of glutathione S-transferase (GST) isozymes, are important in the context of cancer. However, the specific impacts of these genetic factors on brain cancer prognosis requires further investigation.[7]

The tumor suppressor protein 53 (p53) plays a crucial role in preventing tumorigenesis by regulating cellular processes. While associations between GST isozymes and p53 protein expressions are noted in some cancers,[8] their relationship in brain cancer remains poorly understood.

Given the limited knowledge on the interplay between glutathione S-transferase-Mu (GST-M) isozyme, p53 protein expressions, lifestyle factors, and the prognosis of brain tumors, this study aims to explore the expression profiles of GST-M isozyme and p53 protein in both healthy and tumorous brain tissue. The results will be compared with clinical features and lifestyle factors to identify any correlations or associations that may exist.

MATERIALS AND METHODS

Study Design and Recruitment of Participants

This study involved a retrospective analysis of clinical data from patients with intracranial tumors treated at the Health Sciences University Kecioren Training and Research Hospital Neurosurgery Clinic from 2017 to 2019. Patients who met the inclusion and exclusion criteria were selected through archival sampling. The study ultimately recruited 149 subjects, ranging in age from 6 to 83 years, with a gender distribution of 62 females and 87 males, making the sample representative of the broader population. Histopathological analysis of tumor and adjacent healthy tissues was performed using immunohistochemical (IHC) staining to profile GST-M and p53 protein expression.

Inclusion and Exclusion Criteria

The inclusion criteria encompassed patients with intracranial malignancies, including both primary and secondary brain cancers, who had both tumor and adjacent healthy tissue samples available for study.

To ensure the validity of the study, exclusion criteria were established to exclude patients who were unable to provide informed consent, had undergone prior targeted therapy for the p53 or GST-M pathways, presented with initial tumors outside the intracranial region, provided insufficient tumor samples, or had severe comorbid diseases.

Ethics Clearance and Participant Consent

The institutional review board at Health Sciences University Kecioren Training and Research Hospital approved this study (No: 2012-KAEK-15/1810, dated: 27. 02. 2019). Participants were provided with detailed information about the study, and written informed consent was obtained from each.

Data Collection

Demographic and clinical data were systematically collected through a comprehensive checklist that included age, gender, smoking and alcohol habits, prior exposure to radiotherapy (RT) and CT, surgical history, affected brain region, resection margins, lesion localization, and postoperative status. This data was retrospectively acquired to provide a thorough background analysis of the subjects.

Tumor and adjacent healthy tissues were collected from surgical sites by experienced neurosurgeons following standardized procedures. These samples were then embedded in paraffin for subsequent analysis. The expression of GST-M and p53 genes was meticulously assessed using IHC methods, with expression levels classified into categories (0, 1, 2, or 3)

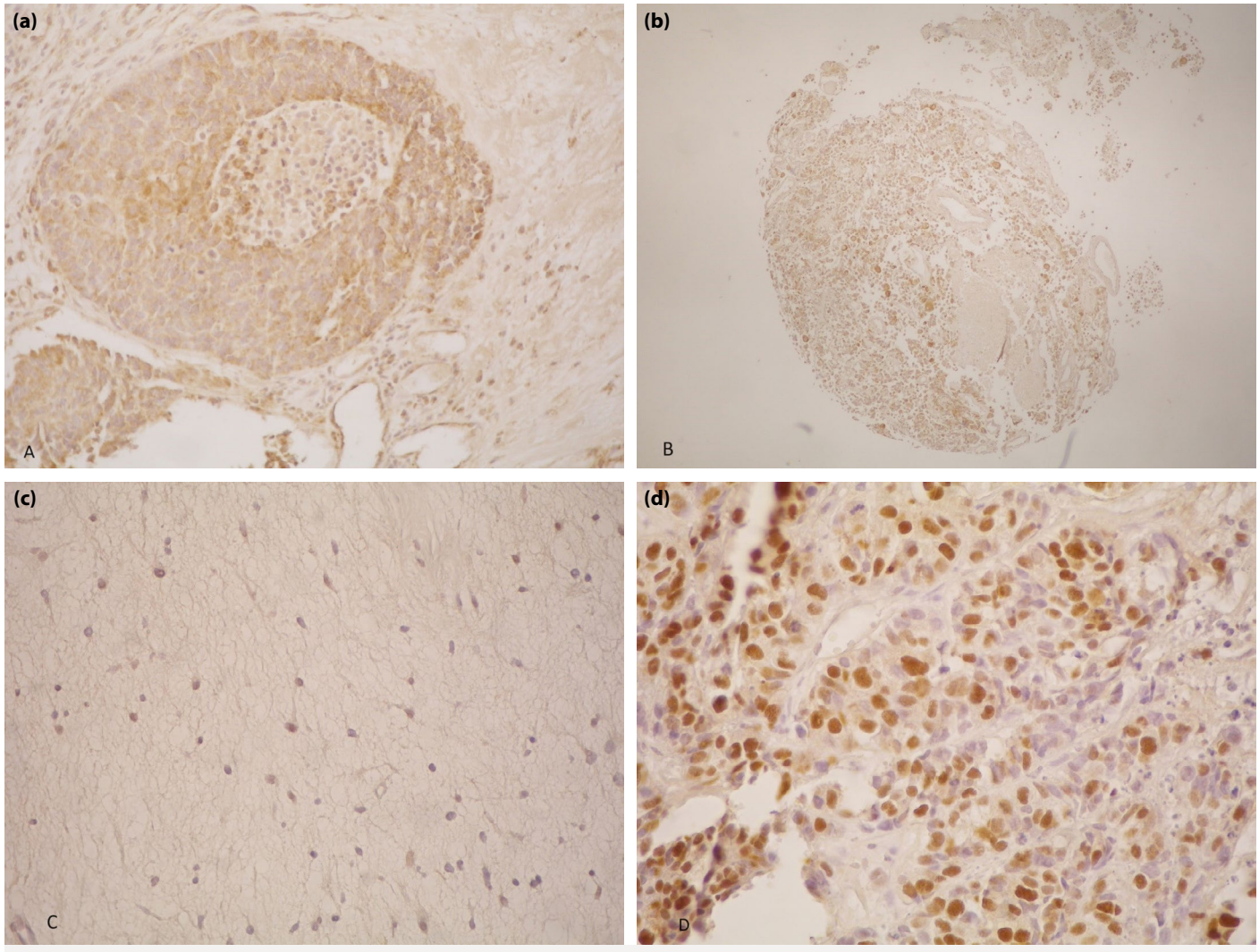

Figure 1. Immunohistochemical staining of tumorous tissue. **(a)** GST-M (+) positive nuclei stained brown at x20 magnification. **(b)** GST-M (+) positive nuclei stained brown at x4 magnification. **(c)** p53 (-) negative nuclei, unstained, at x40 magnification. **(d)** p53 (+) positive nuclei stained brown at x40 magnification.

based on microscopic examination after immunostaining, providing a precise evaluation of expression levels.

Histopathological Examination

Histopathological analysis of cerebral neoplastic tissue is essential for gaining detailed insights into tumor characteristics, which supports diagnosis, categorization, and prognosis. This procedure initiates with the procurement of tissue during surgical resection, followed by its immersion in 10% buffered formalin to ensure preservation. The tissue is then embedded in paraffin wax, and thin sections (4 µm) are prepared using a microtome. Glass slides are then used for staining with hematoxylin and eosin to analyze morphology, as well as for IHC staining to detect GST-M and p53 gene expression. This comprehensive analysis enables informed therapeutic decisions and prognostic predictions.

Immunohistochemical (IHC) Staining

For immunohistochemistry, the tissue sections were initially soaked in a 1% hydrogen peroxide (v/v) solution in methanol at room temperature for 10 minutes to neutralize natural peroxidase activity. Following this, the sections were rinsed in distilled water for five minutes. The extraction of GST-M and p53 proteins was then performed using a 0.01 M citrate buffer (pH 6.0) in a household pressure cooker. After another rinse in distilled water, the sections were immersed in a 0.05 M Tris-HCl solution (pH 7.6) with 0.15 M sodium chloride. To minimize nonspecific background staining, the sections were

treated with Super Block (streptavidin/HRP complex [SHP125]; ScyTek Laboratories, USA) for 10 minutes at room temperature. The sections were then incubated overnight at 4°C after treatment with primary antibodies, diluted at ratios of 1:1,000 for anti-GST-M and 1:50 for anti-p53. Anti-GST-M was sourced from Boster Biological, Pleasanton, CA, USA, and anti-p53 was obtained from Santa Cruz Biotechnology Inc., USA. Following a 15-minute wash in tris-buffered saline, the sections were treated with a biotinylated link antibody and SHP125 at room temperature. Aminobenzidine was used to visualize the peroxidase activity within the tissues. The nuclei were faintly counterstained with hematoxylin, after which the slices were dried and mounted. Each sample involved an independent examination of tissue nuclei from the invasive front and the central region of the tumor, focusing specifically on the nuclear and cytoplasmic staining of tumor epithelial cells (Fig. 1). Staining intensity was evaluated on a scale of 0 (no staining), 1 (poor staining), 2 (moderate staining), and 3 (strong staining).[9,10]

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA). Continuous data were presented as mean±standard error of the mean (SEM), while categorical data were expressed as frequency (n) and relative frequency (%). The IHC staining profiles of tumor and normal tissues were compared, and the scores were analyzed based on the demographic and clinical characteristics of the patients. Staining scores for IHC analysis were derived from the intensity of positive staining observed in tumor tissues. Normality was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests, while homogeneity of variance was tested using Levene's test. Since the data did not conform to the assumptions of a normal distribution, non-parametric tests were employed in the analysis. The Mann-Whitney U test was utilized to compare differences between two independent groups, while the Kruskal-Wallis H test was used to assess differences among more than two independent groups. The Chi-squared test was applied for comparing categorical data, and Fisher's exact test was used when more than 20% of cells had expected frequencies below 5. Spearman's rank correlation coefficients were calculated to determine correlations between expressions and continuous clinical and demographic data. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The current study included a cohort of 149 individuals with brain tumors and adjacent normal tissue samples. The patients had a mean age of 49.44±8.09 years, ranging from 6 to 83 years. An analysis of clinical data from these 149 brain tumor patients showed that the cohort comprised 87 males (58.4%) and 62 females (41.6%). Additionally, based on patient information and the "Tumor, Nodes, Metastases" (TNM) classifications, the patients were categorized into different tumor grades. A significant portion of the cohort, comprising 88 individuals (59%), was distributed across three grades: 32 patients (21.5%) were classified as grade 1, 18 patients (12%) as grade 2, and 27 patients (18.1%) as grade 3. The remaining 61 cases (40.9%) displayed either metastatic characteristics or malignancies that precluded grading. Of the total cohort, a majority of 105 patients (70.5%) with a male to female ratio of 59:46 presented with primary tumor types, while a smaller subset of 44 individuals (29.5%) exhibited secondary tumor types.

Table 1. Demographic attributes of patients

	Patient group (n=149)	
	n	%
Demographic data		
Gender		
Female	62	41.6
Male	87	58.4
Age (years) mean±SD	49.44±8.09	
Age distribution		
<30	25	16.8
30–45	31	20.8
46–60	43	28.9
>60	50	33.5
Habits		
Alcohol consumption		
Yes	15	10.1
No	134	89.9
Smoking		
Yes	45	30.2
No	104	69.8

SD: Standard deviation. Total number of patients: 149. Categorical data are presented as the number of patients (n) and the corresponding percentage (%).

Regarding lifestyle factors, it was noted that 45 patients (30.2%) had a history of smoking until the day of diagnosis, while the majority, 104 patients (69.8%), had never smoked. Additionally, 15 patients (10.1%) reported alcohol use, while the vast majority, 134 patients (89.9%), did not consume alcohol. The treatment history of the patients revealed that 55 (36.9%) received RT, while 32 (21.5%) underwent CT. To provide a comprehensive overview, the demographic details and treatment history of the patients, as well the clinical profile of the patient cohort, are presented in Table 1 and Table 2, respectively.

Table 2. Clinical profile of the patient cohort

Clinical data	Patient group (n=149)		Clinical data	Patient group (n=149)	
	n	%		n	%
Chemotherapy			Lesion localization		
Yes	32	21.5	Frontal	43	28.9
No	116	77.9	Parietal	20	13.4
Missing	1	0.7	Temporal	17	11.4
Radiotherapy			Cerebellar	15	10.1
Yes	55	36.9	Other locations	54	
No	92	61.7	Hypophysis	7	4.7
Missing	2	1.3	Sella	7	4.7
Tumor type			Frontobasal	6	4.0
Primary	105	70.5	Occipital	6	4.0
Secondary	44	29.5	Frontotemporal	5	3.4
Tumor location			Frontoparietal	4	2.7
Bilateral	1	0.7	Parietooccipital	4	2.7
Middle	24	16.1	Posterior fossa	4	2.7
Left	66	44.3	Lateral ventricle	3	2.0
Right	58	38.9	Cerebellopontine angle	3	2.0
Number of surgeries			Temporoparietal	2	1.3
1	113	75.8	4th ventricle	1	0.7
2	30	20.1	Intraventricular	1	0.7
3	2	1.3	Parafalxian	1	0.7
4	1	0.7	Postoperative status		
5	1	0.7	Alive	97	65.1
7	1	0.7	Exitus	52	34.9
10	1	0.7			

Total number of patients: 149. Categorical data are presented as the number of patients (n) and the corresponding percentage (%).

The majority of participants underwent primary surgical intervention (75.8%), followed by a second round of surgical procedures (20.1%). Notably, 44.3% of the subjects presented with tumors located in the left hemisphere of the brain, while 38.9% exhibited tumors in the right hemisphere. A smaller proportion, namely 16.1% and 0.7% of the subjects, displayed tumors in the middle and bilateral regions, respectively. An analysis of lesion distribution revealed that the frontal section accounted for the highest frequency at 28.9%, followed by the parietal region at 13.4%, the temporal region at 11.4%, and the cerebellar region at 10.1%. The remaining cases (36.4%) displayed a diverse range of tumor localizations, as detailed in Table 2. The overall postoperative survival rate was found to be 65.1%.

No significant correlations were observed between age and the number of surgeries, and the expressions of p53 and GST-M proteins in either normal or tumor tissues (p>0.05).

Table 3 presents the frequency distribution of IHC staining categories for GST-M and p53 proteins in both brain tumor and adjacent normal tissues.

Strong staining of GST-M was not observed in either tumorous or healthy tissues. However, a moderate degree of GST-M expression was detected in 11 (7.4%) brain tumor tissues, compared to only 3 (2.0%) instances in normal tissues. Furthermore, GST-M exhibited weak expression in 42 (28.2%) tumor tissues and in 17 (11.4%) normal tissues. The observed staining intensity distribution of GST-M expression

Table 3. Frequency distribution of immunohistochemical staining scores for GST-M and p53 proteins in brain tumor and adjacent normal tissues

Staining scores	GST-M				p53			
	Tumor		Normal		Tumor		Normal	
	n	%	n	%	n	%	n	%
0	96	64.4	129	86.6	117	78.5	145	97.3
1	42	28.2	17	11.4	32	21.5	4	2.7
2	11	7.4	3	2.0	–	–	–	–
p-value	<**0.0001**				<**0.0001**			

Staining scores were assigned based on the intensity of positive staining in tumor tissues. The staining intensities are categorized as follows: 0: negative expression, +1: weak expression, +2: moderate expression. Categorical data are presented as the number of patients (n) and the corresponding percentage (%). p<0.0001, as determined by the Mann-Whitney U test, indicates a statistically significant difference compared to the normal group.

demonstrates a substantial distinction between tumorous and healthy tissues (p<0.0001), with approximately a 2.6-fold higher frequency in tumor tissues. In contrast, robust p53 expression remained absent in both normal (97.3%) and tumor (78.5%) tissues. It is important to highlight that although p53 expression levels were low in both normal (2.7%) and tumor tissue samples (21.5%) based on the overall unstained samples, a significantly higher incidence of these low-expression samples was observed among the cancer patient groups (p<0.0001). Despite the lack of strong or moderate p53 expression, tumor tissues displayed a frequency of p53 expression nearly seven times greater than that observed in normal tissues, indicating a significant disparity. The impact of factors such as gender, age, smoking, alcohol consumption, and RT and CT status on expression levels were also investigated (Table 4). The differences in the observed frequencies of GST-M and p53 protein expression between females and males did not reach statistical significance (p>0.05) in either tumor or control samples. Similarly, no statistically significant differences were found regarding the age of the subjects and the protein expression levels of GST-M and p53 (p>0.05).

Expression levels of GST-M and p53 in tumor tissues of patients who underwent RT or CT showed no statistically significant differences (p>0.05). While RT or CT treatments did not significantly affect GST-M expression in normal tissues, a significantly higher expression of p53 was observed in the normal tissues of patients who received CT compared to those who did not (p=0.01).

The expression of the p53 protein in secondary-type tumor tissues was markedly higher compared to primary tumors (p=0.004). In contrast, no significant differences were observed in GST-M expression between primary and secondary subjects (p>0.05).

This investigation into the distribution of GST-M and p53 expression levels in brain tumor tissues offers valuable insights, especially in the context of smoking and alcohol consumption patterns. Intra-group comparisons of healthy and tumorous tissues from smokers and non-smokers, as well as alcoholic and non-alcoholic individuals, revealed no significant differences in the expression frequencies of both GST-M and p53 proteins (p>0.05).

The expressions of GST-M and p53 in tumor tissues across different localizations were also analyzed, with detailed results presented in Table 5. No significant variations were observed in the expression patterns of tissues based on tumor localizations (p>0.05). Similarly, in both normal and tumorous tissues, no significant differences were detected in the frequency of GST-M and p53 expression when considering the specific regions of the brain affected by cancer (p>0.05). Moreover, the postoperative survival outcomes in CNS tumors appeared to be independent of the expression levels of GST-M and p53 (p>0.05). Of the study participants, 52 subjects died post-operation, with 94.2% of them having had high-grade primary tumors or metastatic malignancies.

DISCUSSION

This study provides new insights into the complex interactions between GST-M and p53 expression levels, clinical features, tumor prognosis, and demographic and lifestyle factors. It specifically examines factors such as age, gender, substance use (alcohol and tobacco), tumor location, and histopathology in a Turkish population of brain tumor patients, aiming to understand their impact on tumor development, prognosis, and survival outcomes.

In this cohort, the mean age at diagnosis was 49.44 years, compared to a previous study in Turkish patients that reported a mean age of 46.72 years.[11] By contrast, data from European and American populations show mean ages of 53.24 and

Table 4. GST-M and p53 expression in brain tumor and adjacent normal tissues, analyzed by demographic and clinical data

Variables	GST-M		p53	
	Tumor	Normal	Tumor	Normal
Demographic data				
Gender				
Female	0.54±0.09[a]	0.15±0.06[a]	0.20±0.05[a]	0.03±0.02[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
Male	0.36±0.06[a]	0.16±0.04[a]	0.22±0.04[a]	0.02±0.02[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
p-value	0.073	0.699	0.785	0.667
Age				
<30	0.20±0.10[a]	0.12±0.07[a]	0.36±0.10[a]	0.04±0.04[a]
	(0–2)[b]	(0–1)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
30–45	0.48±0.12[a]	0.19±0.09[a]	0.23±0.08[a]	0.03±0.03[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
46–60	0.47±0.10[a]	0.09±0.06[a]	0.21±0.06[a]	
	(0–2)[b]	(0–2)[b]	(0–1)[b]	–
	0.00[c]	0.00[c]	0.00[c]	
>60	0.48±0.09[a]	0.20±0.06[a]	0.14±0.05[a]	0.04±0.03[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00c	0.00[c]	0.00[c]	0.00[c]
p-value	0.194	0.463	0.188	0.636
Smoking				
Yes	0.42±0.09[a]	0.16±0.06[a]	0.24±0.06[a]	0.04±0.03[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
No	0.44±0.06[a]	0.16±0.04[a]	0.20±0.04[a]	0.02±0.01[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
p-value	0.902	0.972	0.583	0.389
Alcohol consumption				
Yes	0.53±0.13[a]	0.27±0.12[a]	0.27±0.12[a]	0.07±0.07[a]
	(0–1)[b]	(0–1)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]
No	0.42±0.06[a]	0.14±0.04[a]	0.21±0.04[a]	0.02±0.01[a]
	(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
	0.00[c]	0.00[c]	0.00[c]	0.00[c]

Table 4 (cont). GST-M and p53 expression in brain tumor and adjacent normal tissues, analyzed by demographic and clinical data

Variables		GST-M		p53	
		Tumor	Normal	Tumor	Normal
	P-value	0.257	0.134	0.618	0.320
Clinical data					
CT					
	Yes	0.45±0.10[a]	0.21±0.08[a]	0.33±0.08[a]	0.09±0.05[a]*
		(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
		0.00[c]	0.00[c]	0.00[c]	0.00[c]
	No	0.42±0.06[a]	0.14±0.04[a]	0.18±0.04[a]	0.01±0.01[a]*
		(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
		0.00[c]	0.00[c]	0.00[c]	0.00[c]
	p-value	0.522	0.362	0.061	0.010
RT					
	Yes	0.42±0.08[a]	0.11±0.04[a]	0.21±0.05[a]	0.04±0.02[a]
		(0–2)[b]	(0–1)[b]	(0–1)[b]	(0–1)[b]
		0.00[c]	0.00[c]	0.00[c]	0.00[c]
	No	0.43±0.07[a]	0.18±0.05[a]	0.22±0.04[a]	0.02±0.02[a]
		(0–2)[b]	(0–2)[b]	(0–1)[b]	(0–1)[b]
		0.00[c]	0.00[c]	0.00[c]	0.00[c]
	p-value	0.910	0.383	0.921	0.625

Staining scores were determined based on the intensity of positively stained tumor tissues. The staining intensities are categorized as follows: 0: negative expression, +1 weak expression, +2: strong expression. Categorical data are expressed as the number of samples (n) and their percentage (%). a: Mean±SEM; b: Range of staining intensity (minimum to maximum); c: Median. (*) p<0.05 is considered statistically significant. The Mann-Whitney U test is used for comparing two independent groups and the Kruskal-Wallis H test is applied when analyzing more than two independent groups. CT: Chemotherapy; RT: Radiotherapy.

60.16 years, respectively, indicating that brain tumors in Türkiye are typically diagnosed in middle-aged individuals. This discrepancy may point to differences in epidemiology, risk factors, or healthcare practices compared to other regions.

Both genders are affected by all types of CNS tumors. Data from the Central Brain Tumor Registry of the United States indicates the distribution among all primary brain tumors consists of 42% females and 58% males.[12] Our study's gender distribution aligns with this global rate; however, our clinical observations show that males generally have a higher incidence of brain tumors, with the exception of meningiomas, which are more prevalent among females. Additionally, glioblastoma demonstrates higher incidence and mortality rates in males, consistent with findings from existing brain tumor epidemiology studies.[13,14]

Neuroepithelial tumors represent the largest group of primary brain tumors, followed by meningiomas and pituitary adenomas. In the United States, meningiomas account for about 50% of all nonmalignant tumors, making them the most common nonmalignant brain and CNS tumors. Conversely, gliomas, especially glioblastoma, are the most prevalent malignant tumors.[15] Epidemiological studies have noted significant variations in the incidence rates of neuroepithelial tumors based on country, race, age at diagnosis, gender, and histological type.[16] Within the Turkish population, neuroepithelial tumors account for approximately half of all primary malignancies, with a higher prevalence observed in males across lla ge groups.

Studies indicate that the majority of intracranial tumors in adults are located supratentorially, with the most common sites being the frontal, temporal, and parietal lobes.[17] Our findings are in partial agreement with the existing literature.[17] Few studies have investigated the distribution of brain tumors based on laterality, and no significant differences have been observed in tumor localization between the right and left sides of the central nervous system.[17,18] Our study has yielded results consistent with these findings regarding tumor localization.

Table 5. GST-M and p53 expressions in brain tumor tissues analyzed by lesion localizations

Lesion localizations	GST-M							p53				
	0		1		2		X^2-value, p-value	0		1		X^2-value, p-value
Frontal	24	16.1	15	10.0	4	2.6	4.340[a], 0.839	34	22.8	9	6.0	2.985[a], 0.568
Parietal	13	8.7	6	4.0	1	0.6		13	8.7	7	4.7	
Temporal	13	8.7	4	2.6	0	0.0		15	10	2	1.3	
Cerebellar	9	6.0	5	3.3	1	0.6		12	8.0	3	2.0	
Others	37	24.8	12	8.0	5	3.3		44	29.5	11	7.4	
Total	**96**	**64.4**	**42**	**28.2**	**11**	**7.4**		**117**	**78.5**	**32**	**21.5**	

Categorical data are expressed as the number of samples (n) and relative frequency (%). (*) p<0.05: statistically significant, a: Fisher's exact test (exact significance, two-sided).

We found significant differences in the expression of GST-M and p53 proteins between brain tumor tissues and adjacent normal tissues. It is noteworthy that the expression of GST-M and p53 does not significantly impact postoperative survival in CNS tumors, suggesting that other factors play a more critical role in the prognosis and survival of CNS tumor patients. Previous studies have often associated GST and p53 variants with prognosis.[8,19] Specifically, TP53 mutations are commonly observed in glioblastoma multiforme, the most common and aggressive primary brain tumor.[8] Additionally, another study linked GSTP1 Ile105Val genetic variations with the prognosis of glioblastoma patients.[19] To further elucidate the relationship between GST genetic variations, particularly GST-M, and brain tumor prognosis, we recommend conducting a comprehensive molecular study. This could provide valuable insights into how GST genetic variability influences clinical outcomes in brain cancer patients.

It is important to note that this study is subject to certain limitations. First and foremost, the tumor classification in this study partially deviated from the World Health Organization's classification of CNS tumors.[20] Due to the limited occurrence of certain categories, statistical comparison was not feasible. Consequently, groups with fewer samples were amalgamated into a larger group for inclusion in the statistical analysis. Secondly, the retrospective nature of the study limited the evaluation of demographic and clinical characteristics, posing challenges in gathering comprehensive and diverse demographic data and increasing the risk of biased information, which could affect the depth and breadth of the analysis. Thirdly, the inclusion of single-center data from a predominantly Turkish population may affect the generalizability of the findings and introduce biases. Fourth, the decision to include 149 patients with brain tumors was made after careful consideration of several factors, particularly the prevalence of the condition in our target population and the availability of tissue samples from participants. It is important to emphasize that our study represents an initial exploration into this area. The smaller sample size was chosen to balance feasibility and resource constraints. Finally, the inclusion of diverse intracranial tumor types, encompassing both primary and metastatic neoplasms, presents another layer of complexity. Consequently, a collaborative multicenter study focusing on specific brain tumor subtypes is essential. This approach would yield a more comprehensive perspective, enhance generalizability, and overcome the limitations associated with single-center data and the heterogeneity of tumor types.

CONCLUSION

This study highlights significant differences in GST-M expression between healthy and tumorous brain tissues, suggesting its role in brain tumorigenesis. Nevertheless, this expression was not linked to clinical features or lifestyle risk factors and did not affect postoperative survival rates. Further research is needed to uncover the underlying mechanisms of these observations in the development and progression of brain cancer.

Ethics Committee Approval: The Health Sciences University Kecioren Training and Research Hospital Ethics Committee granted approval for this study (date: 27.02.2019, number: 2012-KAEK-15/1810).

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