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Chemoresistance in Malignant Intracranial Tumors: Longer Survival with Negative MDR1 Expression

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ABSTRACT

AIM: To analyze the Glutathione S-transferase (GST)-P, GST-M, cytochrome p450 (CYP)1-A1, CYP1-B1, and multidrug resistance (MDR)-1 expressions in malignant intracranial tumor (ICT)s, and to elicit their role on patient survival.

MATERIAL and METHODS: GST-P, GST-M, CYP1-A1, CYP1-B1, and MDR-1 expressions were analyzed using immunostaining in 149 samples from 141 patients with preoperative ICT diagnosis. The case characteristics were reviewed, and the enzyme expressions were equated based on the age, gender, and tumor type. Then, 77 of 141 patients with malignant ICT and complete medical records postoperative were also investigated in detail for the relationship between the diagnosis, enzyme expression, and overall survival.

RESULTS: The average age was 49.44 years, with 83 (58.45%) male patients. Among the 77 malignant ICTs, 38 (49.3%) and 29 were glial tumors and metastases, respectively, with a 13.35-month overall survival. Patients with metastatic tumor have approximately threefold higher GSTP level than those with glial tumors. MDR-1 expression was approximately twofold higher in > 60-year-old patients. No statistically significant association was found between patients' smoking behaviors, alcohol consumption, and overall survival. Only MDR-1 expression was correlated with overall survival. Better overall survival was observed in patients with a negative MDR-1 expression than those with a positive one.

CONCLUSION: MDR-1 is an important indicator of survival in malignant intracranial tumor patients. Longer survival is associated with negative MDR-1 expression.

KEYWORDS: Brain, Tumor, Glutathione S-transferase, Cytochrome p450, Multidrug resistance

INTRODUCTION

The most common malignant intracranial tumors (ICTs) are metastases and gliomas with limited surgical treatment success. Other treatment methods are challenging due to many factors, including chemo- and radioresistance. Chemotherapeutic resistance, being a major problem in cancer treatment, restricts the efficacy of multidrug protocols in advanced malignancies and malignant ICTs. Intracellular drug inactivation or metabolism is one of the chemoresistance mechanisms.

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Glutathione S-transferase (GST), cytochrome p450 (CYP), and multidrug resistance (MDR) are important human body tumor chemoresistance indicators. GST and CYP enzymes might have a role on chemotherapy inactivation against malignant neoplasms (1,21). Fifty percent of all gliomas are glioblastomas (GBM) and extremely locally invasive tumors. Even though alkylators are widely utilized in the systemic drug therapy of GBMs, chemotherapeutic resistance remains an obstacle on the management of these neoplasms (2,15,17). The most significant phase I drug degradation enzymes are CYPs, which activate various types of carcinogenic substances (1,3). 4-Hydroxylation of estrogens is catalyzed by CYP1B1, which has an important role in hormonal carcinogenesis and is overexpressed in various kinds of cancer, including liver, breast, lung, intestinal, and urogenital systems. This specific protein cannot be found or are less expressed in normal tissues (12,14). MDR1 (P-glycoprotein) is a medicine carrier protein that is expressed in some parts of the human body especially in the blood–brain and blood–testis barriers. Many drugs, including antineoplastic agents, anti-arrhythmic medicines, and HIV protease enzyme and proton pump inhibitors, are associated with MDR1 (18). In the 1980s, the MDR1 gene expression has been widely investigated *in vitro* from its cloning to its clinical relevance (18). But research on the expression of GST, CYP, and MDR1 and their relationship with survival in malignant ICTs is scarce. To identify potential indicators for the survival of malignant ICT patients, we conducted a prospective clinical study in 141 patients with 149 specimens. Seventy-seven had malignant ICTs and were investigated in detail based on demographic characteristics, smoking, alcohol consumption, detoxifying enzyme expression, and overall survival rates. We focused on GSTP, GSTM, CYP1A1, CYP1B1, and MDR1 expressions in the distinct groups of malignant ICTs. Possible correlations between the enzymes, tumor types, and overall survival were analyzed.

MATERIAL and METHODS

The data of patients who underwent ICT surgical treatment between 2016 and 2018 in the Department of Neurosurgery have been recorded and statistically analyzed toward the end of March 2019.

The dataset includes patients' demographic characteristics, histopathological examinations, expressions of enzymes, and follow-ups. This prospective study has been approved by the Kecioren Research and Education Hospital Ethics Committee (1267/28.12.2016). Written informed consent was obtained from the patients or their legal guardians for the analysis of specimens and the use of anonymous clinical data. The same team performed all tumor surgeries based on a standard surgical protocol, while a neurosurgeon performed the clinical data acquisition.

This study has two parts: first, the analysis of all intracranial lesions for GST, CYP, and MDR expressions, and, second, the overall survival analysis of malignant ICT patients based on the different enzyme expressions, smoking and alcohol habits, and sex.

A total of 141 patients underwent intracranial lesion resection in a 33-month period, with 149 samples obtained and analyzed. The study focused on 77 patients with malignant ICTs and registered all medical records and follow-ups. GSTP, GSTM, CYP1A1, CYP1B1, and MDR1 were examined in the neoplastic tissues and investigated in healthy cerebral tissue samples, which were detected and obtained from the tumor-surrounding tissue during the histopathological examination of the specimens.

Histopathological Examination

The histopathological analysis of the surgically removed tissues was performed based on a standard protocol. Firstly, tissues were macro- and microscopically analyzed, having two specimen samples taken by the same pathologist for every examination: a neoplastic tissue sample and a microscopically healthy tissue around the tumor. All the lesions were resected and studied in patients with multiple removable tumors, attempting to achieve the uttermost direct tumor invasion to the neural tissue while removing the blocks. The differentiation level and anaplastic features of tumor, the features of the neoplasm border, and the existence of brain invasion were considered tumor-grading indicators. All pathological features analyzed were sought in each sample, clearly recording their presence or absence.

Immunohistochemical Staining

A 10% buffered formalin was used for the fixation of the specimens embedded in paraffin blocks. After cutting the slices in 4-µm thickness, one of them was dyed with hematoxylin-eosin for tissue structure monitoring. We incubated the slices in 1% hydrogen peroxide (v/v) and methanol for 10 minutes at 20°C–22°C to block endogenous peroxidase activity. Then, the slices were rinsed in purified water for 5 minutes, followed by antigen recovery for 3 minutes using 0.01M citrate buffer (pH 6.0) with an autoclave. After rinsing, the slices were moved to the 0.05M Tris-HCl (pH 7.6), including the 0.15M sodium chloride (TBS). Super block (SHP125) (ScyTek Laboratories, USA) was used to incubate the slices at 20°C–22°C for 10 minutes to block nonspecific background staining. Diluted primary antibodies (1:750 for GSTP; 1:400 for GSTM; 1:250 for MDR1; 1:750 for CYP1A1; 1:750 for CYP1B1) were used to cover the sections in Tris-buffered saline at 4°C for a night (Anti-GSTP (LS-C211876) (Boster Biological, Pleasanton, California, USA); anti-GSTM (NBP2-22186) (Novus Biologicals, Littleton, Colorado, USA); anti-MDR1 (MA1060) (Boster Biological, Pleasanton, California, USA); anti-CYP1A1 (sc-20772) and anti-CYP1B1 (sc-32882) (Santa Cruz Biotechnology Inc, USA)). For 15 minutes, the slices were rinsed in TBS solution and then incubated at 20°C–22°C for biotinylated link antibody (SHP125) (ScyTek Laboratories, USA), utilizing Streptavidin/HRP complex (SHP125) (ScyTek Laboratories, USA) in the following step of the treatment. Peroxidase activity in the specimens was visualized by diaminobenzidine. Hemotoxylin slightly counterstained the nuclei, and then the slices were dried out and assembled, with positive and negative controls in every examination.

Two pathologists, who were not informed about the patients, performed the light microscopic examination of the immunohistochemically stained sections and recorded the immunostaining characteristic data, with the color brown signifying positive staining in the cellular cytoplasms and/or nuclei. Scoring was also performed by the same pathologists, but score differences of the pathologists were corrected by consensus. The intensity of staining was graded as 0 (no staining), 1 (weak staining), and 2 (moderate staining) (Figure 1A-F).

Follow-Up

The follow-up period was until the death of the patient or March 2019, if they survived, except for the ones that were lost to follow-up.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) v.25.0 (IBM Corp., Armonk, NY, USA) was utilized for the statistical analysis of data. Staining scores were compared statistically according to the patients' demographic and clinical characteristics. Shapiro-Wilk test was used for the assessment of normality. The appropriate method was chosen depending on the normality profile and the number of groups. Mann-Whitney U or Kruskal-Wallis test was preferred to determine the differences between the patients' clinical and demographic characteristics and immunohistochemical CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 protein expressions with 95% confidence level. Also, the differences between these protein expressions and the factors were examined using the one-way ANOVA and post hoc Fisher's least significant difference (LSD) test with 95% confidence level for normally distributed groups. Spearman's rank correlation coefficients were used to assess relationships between protein expressions themselves and age. The associations in CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 protein expressions with overall survival were examined using the Kaplan-Meier survival analysis and log-rank test. $p<0.05$ was statistically significant.

RESULTS

First Part of the Study

Among the 141 patients, 83 (58.9%) was male and 58 (41.1%) female, with a mean age of 49.44 (ranging from 6 to 83) years old for the all population, 50.25 (ranging from 6 to 77) in female, and 48.91 (ranging from 11 to 83) in male. Forty-four (31.2%) patients were smokers, while 15 (10.6%) alcohol consumers. Fifty-two (36.9%) patients received radiotherapy (RT), while 30 (21.3%) received chemotherapy (ChT). Ninety-four (66.7%) patients were living at the end of our research. Table I shows the demographic features of the cases, features of the tumors, treatment modalities (RT and ChT), and surgical procedures.

In the whole series, CYP1A1 expression patterns were similar in normal and tumor tissues, with ten (6.7%) tumor tissues and eight (5.4%) normal tissues showing weak CYP1A1 expression. CYP1B1 expression was found to be almost fourfold greater in neoplastic tissues than in the healthy ones. Weak CYP1B1 expression is seen in 62 (41.6%) tumor tissues and 14 (9.4%) normal tissues. Seven (4.7%) tumor tissues and two (1.3%) normal tissues had moderate CYP1B1 expression. Higher expression of GSTM in the tumor tissue than in the

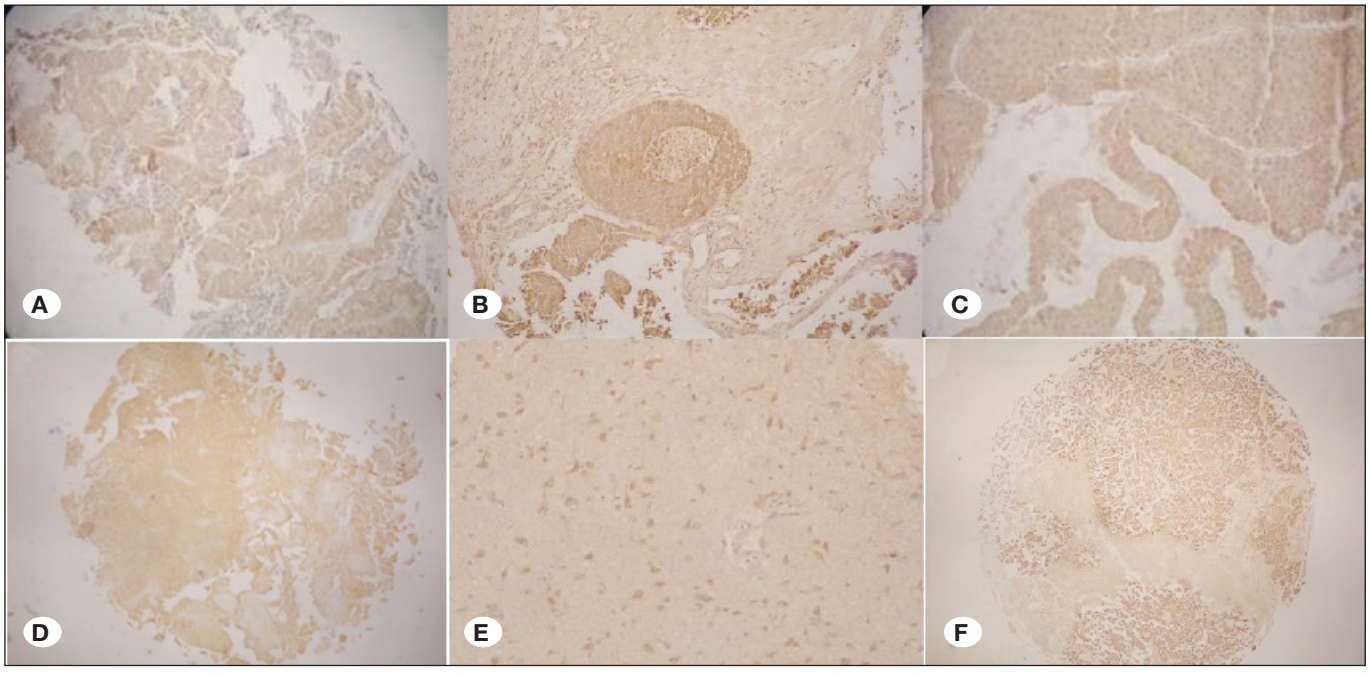

Figure 1: Different enzyme expression patterns are shown using immunohistochemistry. **A)** GSTP weak staining from a patient with metastasis (x10), **B)** GSTM moderate staining from a patient with meningioma grade II (x100), **C)** CYP1A1 weak staining from a patient with pituitary adenoma (x100), **D)** CYP1B1 moderate staining from a patient with medulloblastoma (x40), **E)** MDR weak staining from a patient with GBM (x100), **F)** MDR moderate staining from a patient with metastasis of renal cell carcinoma (x10).

Table I: Demographic and Clinical Characteristics of the Patients

Variable	Number (%)
Sex	
Female	58 (41.1)
Male	83 (58.9)
Total	141 (100)
Age (years)	
<30	22 (15.6)
30-45	30 (21.3)
46-60	42 (29.8)
>60	47 (33.3)
Total	141 (100)
Radiotherapy (RT)	
Yes	52 (36.9)
No	87 (61.7)
No data	2 (1.4)
Total	141 (100)
Chemotherapy (ChT)	
Yes	30 (21.3)
No	110 (78.0)
No data	1 (0.7)
Total	141 (100)
Resection	
Gross Total	91 (61.1)
Partial	5 (3.4)
Subtotal	52 (34.9)
Biopsy	1 (0.7)
Total	149 (100)
Survival	
Alive	94 (66.7)
Died	47 (33.3)
Total	141 (100)
Pathology	
Glial tumor	46 (30.8)
Metastasis	42 (28.2)
Meningioma	32 (21.5)
Pituitary Adenoma	12 (8.0)
Radiation Necrosis	3 (2.0)
Schwannoma	3 (2.0)
Cavernoma	2 (1.3)
Medulloblastoma	2 (1.3)
Central neurocytoma	2 (1.3)
Demyelinating disease	1 (0.7)
DNET	1 (0.7)
Inflammation	1 (0.7)
Craniopharyngioma	1 (0.7)
Lhermitte Duclos Disease	1 (0.7)
Total	**149 (100)**

normal tissue was detected. GSTM expression in tumor tissues was almost threefold higher than the normal tissue with 42 (28.2%) and 17 (11.4%) for weak and 11 (7.4%) and 3 (2.0%) for moderate expression, respectively. The protein expression levels of GSTP were found to be about threefold greater in the neoplastic tissues than the healthy tissues. Weak GSTP expression was observed in 31 (20.8%) tumor tissues and 10 (6.7%) normal tissues. Moderate GSTP expression was found in 5 (3.4%) tumor tissues and 1 (0.7%) healthy tissue. Greater MDR1 expression in the neoplastic tissues than in the healthy tissues was detected (almost tenfold). Weak MDR1 expression is exhibited in 65 (43.6%) tumor tissues and 9 (6.0%) normal tissues. There are 22 (14.8%) tumor tissues and only 1 (0.7%) normal tissue which showed moderate MDR1 expression (Figure 2). GSTM, CYP1A1, and MDR1 expressions were higher in women than in men (Figure 3). CYP1A1 expression was higher in nonsmoker patients; GSTM, GSTP, CYP1B1, and MDR expressions were higher in smoker patients (Figure 4); and GSTM and MDR expressions were higher in non-alcohol consumer patients (Figure 5).

Second Part of the Study

Seventy-seven malignant ICT patients with survival data were included in the second part of the study, with average age of 51.08 years (20 to 82 years). Demographic data (age, sex, smoking habit, and alcohol consumption), tumor characteristics, treatments, and surgical procedures are shown in Table II. There were 54 (70.1%) males and 23 (29.9%) females, with age ranging from 20 to 82 years (mean 51.17 years) and 30 to 76 years (mean 50.87 years), respectively. Twenty-seven (35.1%) of the patients were smokers, and 12 (15.6%) alcohol consumers. Thirty-eight (49.4%) of the patients had glial tumors, 29 (37.7%) had metastatic tumors, and 10 (13.0%) had other types of malignant ICTs. Among the patients with gliomas, 19 (50%) had GBM (grade 4), 7 had anaplastic astrocytoma (grade 3), 4 had oligodendroglioma (grade 2), 3 had diffuse astrocytoma (grade 2), 3 had oligodendroglioma (grade 3), and 2 had gliosarcoma. A total of 50 (64.9%), 43 (55.8%), and 36 (46.8%) patients received RT, local brain RT, and ChT, respectively. Overall survival for the total population was 13.35 months, ranging from 1 to 34: 12.65 months (range: 2–34) for female and 13.65 (range: 1–32) for male. There were 34 (44.2%) of the living patients at the final stage of our research.

When the staining patterns of tumors tissues were analyzed (Figure 6), 5 (6.5%) of the subjects showed weak expression, while 72 (93.5%) showed negative CYP1A1 expression in the tumor tissues. Weak and moderate CYP1B1 expressions

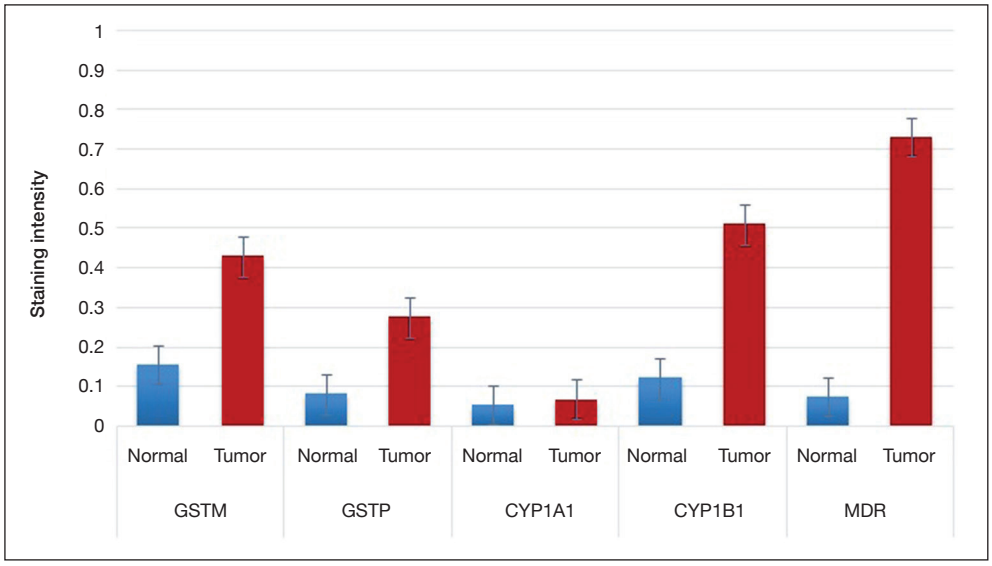

Figure 2: Bar diagram shows the levels of expression of CYP1A1, CYP1B1, GSTM, GSTP and MDR in tumor vs. healthy tissues. All of the enzyme expressions are higher in the tumor samples than the healthy tissue.

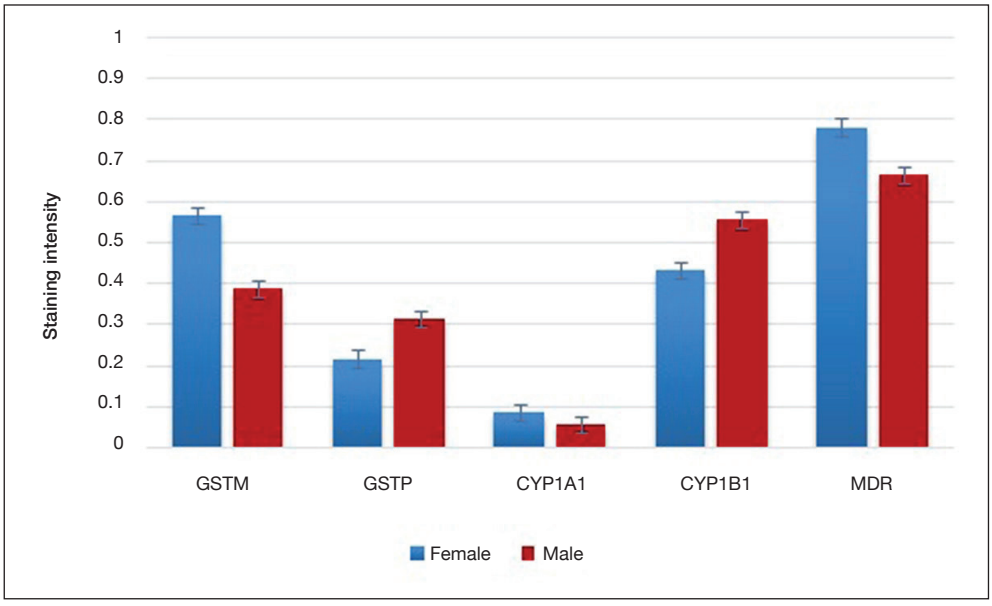

Figure 3: Staining intensity of GSTM, GSTP, CYP1A1, CYP1B1 and MDR in tumor tissues according to the patients' sex. GSTM, CYP1A1 and MDR expressions are higher in female patients while GSTP and CYP1B1 expressions are higher in male patients.

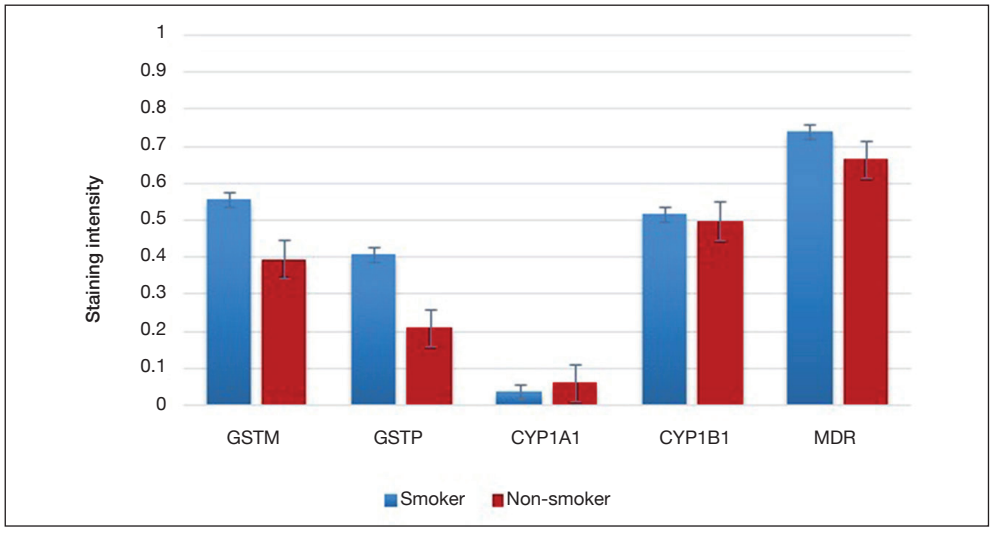

Figure 4: Bar diagram shows staining intensity of GSTM, GSTP, CYP1A1, CYP1B1 and MDR in tumor tissues according to the patients' smoking habits. CYP1A1 expression is higher in non-smoker patients while GSTM, GSTP, CYP1B1 and MDR expressions are higher in smoker patients.

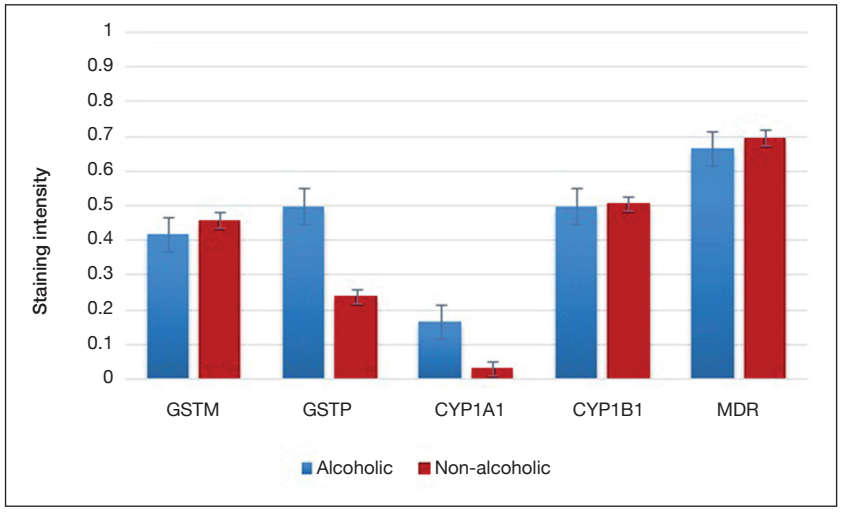

Figure 5: Bar diagram shows staining intensity of GSTM, GSTP, CYP1A1, CYP1B1 and MDR in tumor tissues according to the patients' alcohol consumption habits. GSTM and MDR expressions are higher in non-alcohol consumer patients.

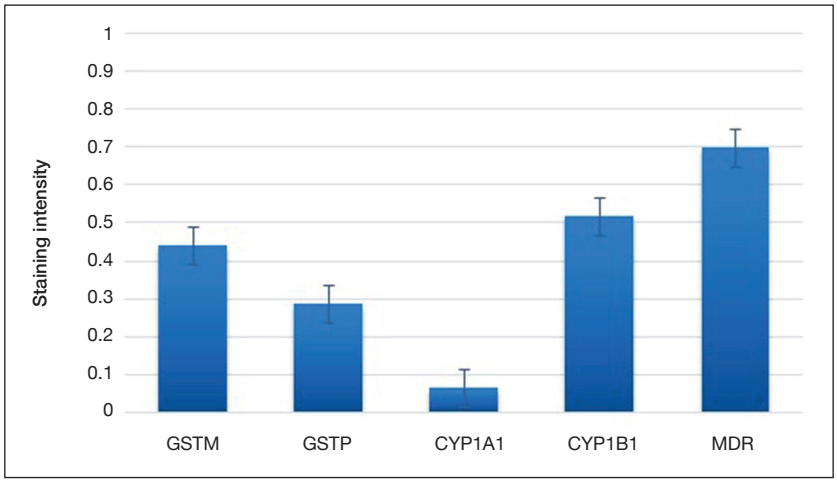

Figure 6: Bar diagram shows the different staining patterns of patients with malignant ICT. MDR expression was higher than the other enzymes.

were found in 32 (41.6%) and 4 (5.2%) neoplastic tissues, respectively. GSTM was weakly and moderately expressed in 24 (31.2%) and 5 (6.5%) tumor tissues, respectively. GSTP expression was identified to be moderate in 2 (2.6%) subjects and weak in 18 (23.4%). Thirty-four (44.2%) of the subjects exhibited weak expression, while 10 (13.0%) of them showed moderate MDR1 expression.

There is a significant correlation between GSTP and CYP1A1 protein expressions with correlation coefficient (r) of 0.311 ($p=0.006$; $p<0.05$). GSTP protein expression moderately correlated with CYP1B1 protein expression ($r=0.383$, $p=0.001$; $p<0.05$), while MDR1 expression significantly correlated with CYP1B1 and GSTP expressions ($r=0.265$, $p=0.020$ and $r=0.253$, $p=0.026$, respectively).

Patients' overall survival was analyzed using the immunohistochemical staining scores of CYP1A1, CYP1B1, GSTP, GSTM, and MDR1 proteins, showing no statistically significant differences between staining scores and the expressions of these five proteins ($p>0.05$).

CYP1A1, CYP1B1, and GSTP expressions were found to be higher in men than women, while GSTM and MDR1 expressions were higher in women. However, the differences in the expression levels of CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 between male and female patients were not statistically significant ($p>0.05$).

MDR1 expression was almost twofold higher in patients older than 60 years old than patients aged 46–60 years, with statistically significant difference ($p=0.041$; $p<0.05$). Except this, no statistically significant correlation and differences were noted between the age of the subjects and protein expression levels of CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 ($p>0.05$).

The expression levels of CYP1A1 decreased almost threefold in patients receiving RT compared to those not receiving RT. The others showed similar expression profiles in both groups. However, the differences between the expression levels of CYP1A1, CYP1B1, GSTM, GSTP, MDR1, and RT status were not statistically significant ($p>0.05$).

The expression levels of CYP1A1 reduced almost threefold in patients receiving ChT compared to those not receiving ChT. However; similar to RT, there were no statistically significant differences in the expression of any of the five proteins between the patients receiving ChT or not ($p>0.05$).

Table II: Demographic and Clinical Data of 77 Patients with Malignant ICT

Variable	Number (%)
Sex	
Female	23 (29.9)
Male	54 (70.1)
Total	77 (100)
Age (years)	
<30	9 (11.7)
30-45	19 (24.7)
46-60	23 (29.9)
>60	26 (33.8)
Total	77 (100)
Smoking	
No	48 (62.3)
Yes	27 (35.1)
Missing	2 (2.6)
Total	77 (100)
Alcohol Consumption	
No	63 (81.8)
Yes	12 (15.6)
Missing	2 (2.6)
Total	77 (100)
Pathology	
Glial tumor	38 (49.4)
Metastasis	29 (37.7)
Other malignant tumors	10 (13.0)
Total	77 (100)
Radiotherapy (RT)	
Yes	50 (64.9)
No	26 (33.8)
Missing	1 (1.3)
Total	77 (100)
Type of RT	
Local brain	43 (55.8)
No	24 (31.2)
Other	6 (7.8)
Missing	4 (5.2)
Total	77 (100)
Chemotherapy (ChT)	
No	39 (50.6)
Yes	36 (46.8)
Missing	2 (2.6)
Total	77 (100)
Resection	
Gross total	49 (63.6)
Subtotal	26 (33.8)
Partial	2 (2.6)
Total	77 (100)
Survival	
Death	43 (55.8)
Alive	34 (44.2)
Total	77 (100)

The differences in GSTP expression between patients with glial, metastatic, and other malignant ICTs were statistically significant (p=0.039; p<0.05), showing about threefold higher GSTP protein level in patients with metastatic tumors than glial tumors (p=0.011; p<0.05). CYP1A1, CYP1B1, GSTM, and MDR1 protein levels were not significantly different in the patients with glial, metastatic, and other malignant tumors (p>0.05).

The associations between CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 expressions and smoking behavior of the patients were analyzed. CYP1B1, GSTM, GSTP, and MDR1 expressions were found higher in smoker group, while GSTP expressions exceed almost twofold in smokers compared to nonsmokers. However, no statistically significant differences were found in the CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 expression levels between smoking and nonsmoking groups (p>0.05).

The associations between CYP1A1, CYP1B1, GSTM, GSTP, and MDR1 expressions and alcohol consumption behavior of the patients were also analyzed. CYP1A1 expression increased in alcohol consumer patients compared to nonconsumers. Alcohol consumption resulted in an almost twofold higher GSTP expression (p=0.045; p<0.05). Expression patterns of CYP1B1, GSTM, and MDR1 were found similar in both alcohol consumer and non-consumer groups, showing no statistically significant differences (p>0.05).

No statistically significant differences were observed between patients with glial, metastatic, other malignant tumors, and their overall survival (Figure 7); patients' smoking behaviors and overall survival (Figure 8); patients' alcohol consumption behaviors and overall survival (Figure 9); overall survival and CYP1A1 expression profile (Figure 10); CYP1B1 expression and overall survival (Figure 11); overall survival and GSTM expression profile (Figure 12); and GSTP expression and overall survival (Figure 13). Patients with negative MDR1 expression had significantly better overall survival rate than those with positive expression both weak and strong (X2= 6.134; p=0.047, log-rank test) (Figure 14).

Overall survival of the patients was also analyzed based on the sex of patients, showing no significant association between the patient sex and overall survival (X2=0.365; p=0.545, log-rank test) (Figure 15).

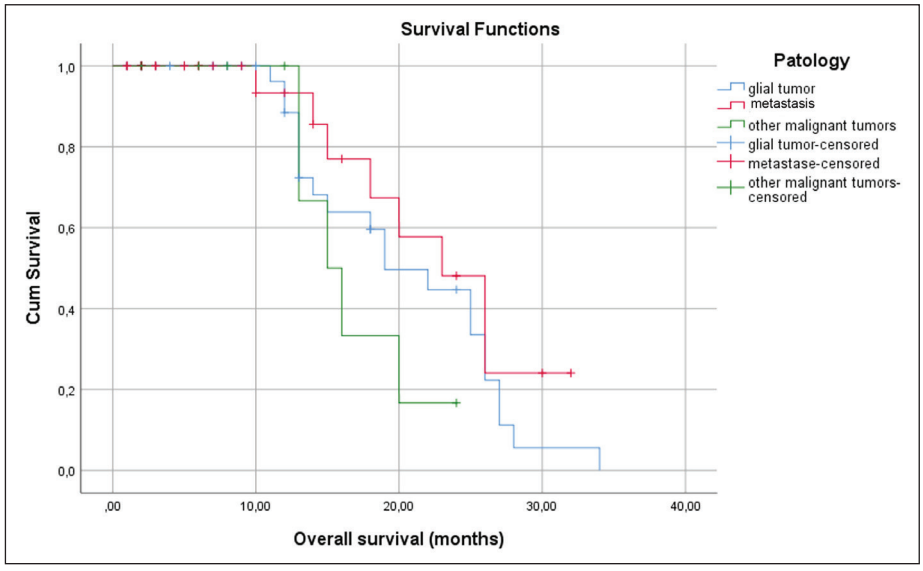

Figure 7: Overall survival of the patients with malignant ICT according to tumor patology. Thirty-eight (49.4%) of the patients had glial tumors, while 29 had metastatic tumors and 10 had other types of malignant ICT. No statistically significant differences were observed between patients with glial, metastatic, other malignant tumors and overall survival (p=0.254).

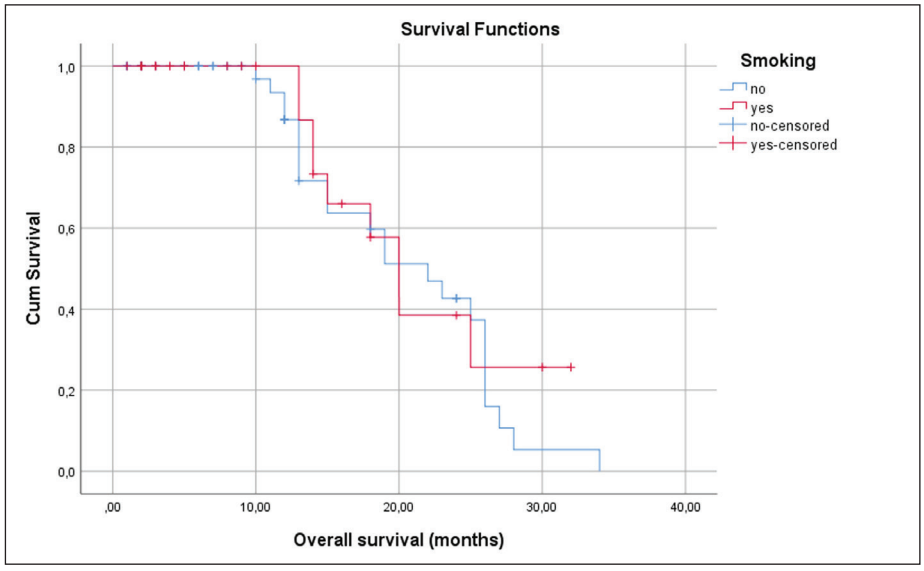

Figure 8: Overall survival of the patients with malignant ICT according to smoking behaviour. Twenty-seven patients were smoker. There is no statistically significant association between patients' smoking behaviours and overall survival (p=0.562).

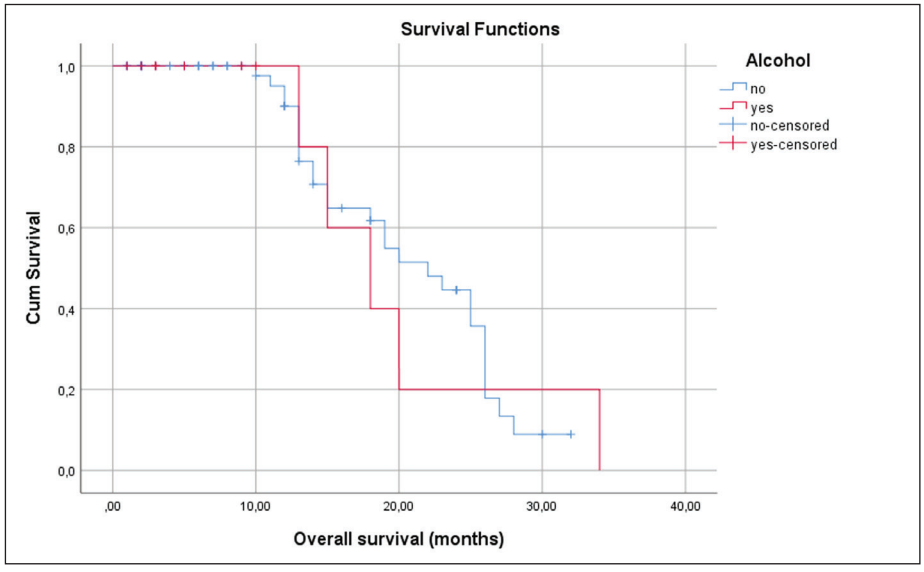

Figure 9: Overall survival of the patients malignant ICT according to alcohol consumption. Twelve patients were alcohol comsumer. There was no statistically significant association between patients' alcohol consumption behaviours and overall survival (p=0.921).

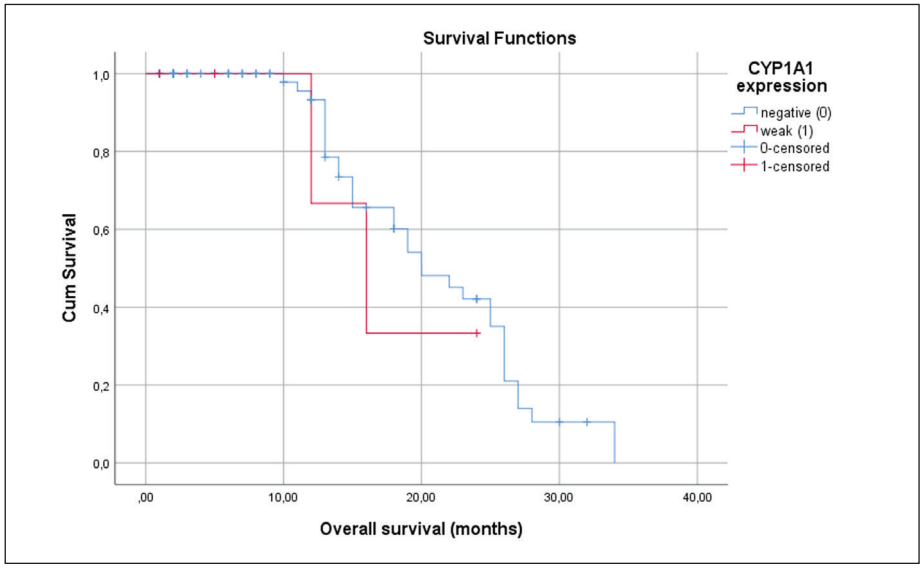

Figure 10: Overall survival of the patients with malignant ICT according to CYP1A1 expression patterns. CYP1A1 expression was detected in 5 (6.5%) patients. There is no statistically significant association between overall survival and CYP1A1 expression profile of patients (p=0.559).

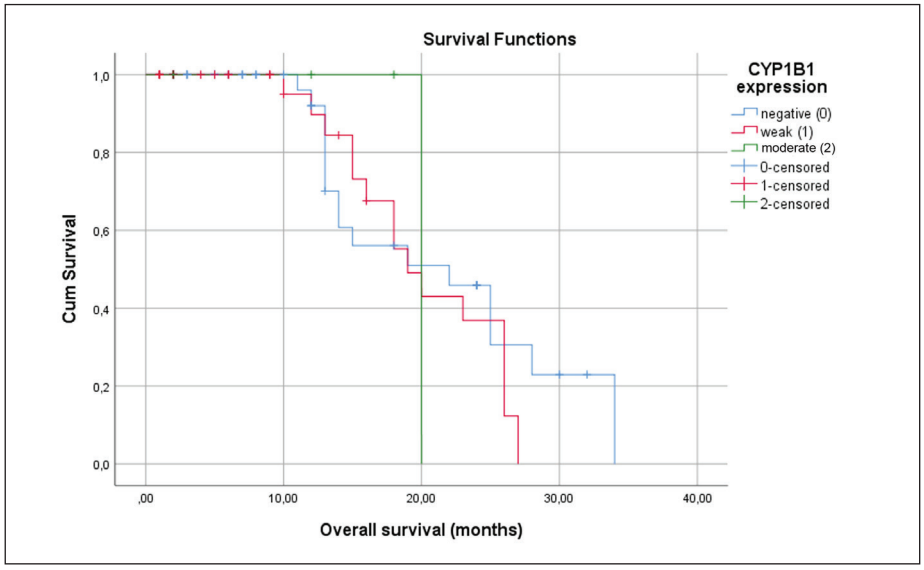

Figure 11: Overall survival of the patients with malignant ICT according to CYP1B1 expression patterns. CYP1B1 expression was detected in 36 (46.8%) patients. There is no statistically significant association between overall survival and CYP1B1 expression profile of patients (p=0.657).

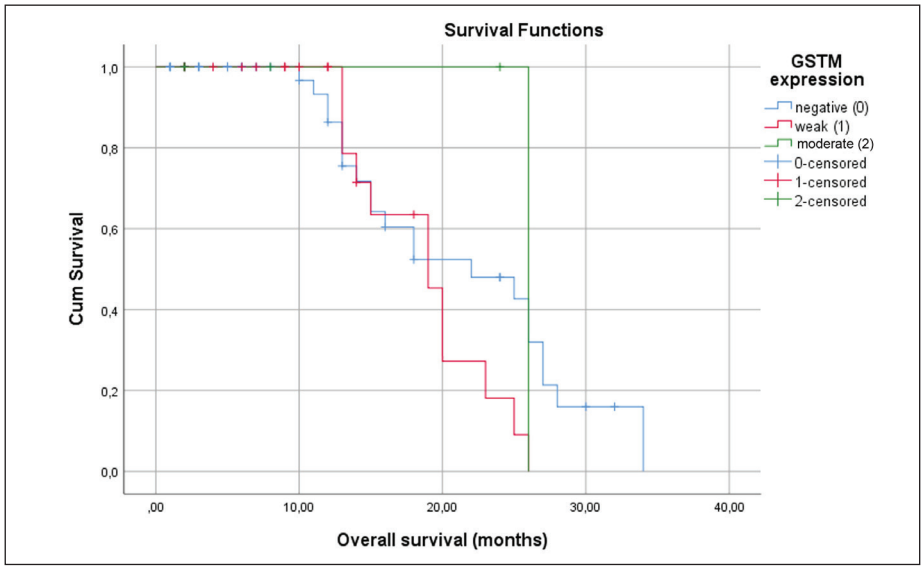

Figure 12: Overall survival of the patients with malignant ICT according to GSTM expression patterns. GSTM expression was detected in 29 (37.7%) patients. There is no statistically significant association between overall survival and GSTM expression profile of patients (p=0.223).

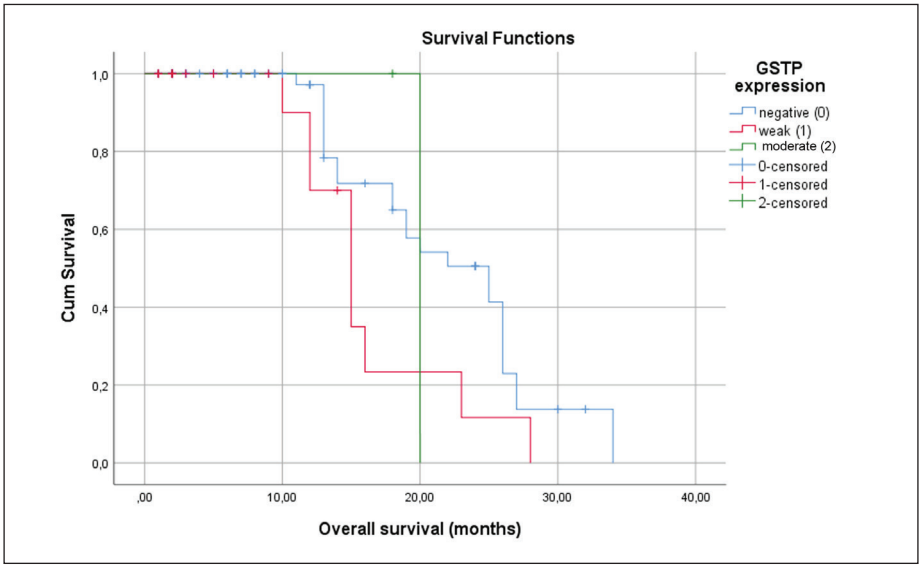

Figure 13: Overall survival of the patients with malignant ICT according to GSTP expression patterns. GSTP expression was detected in 20 (26%) patients. There is no statistically significant association between overall survival and GSTP expression profile of patients (p=0.110).

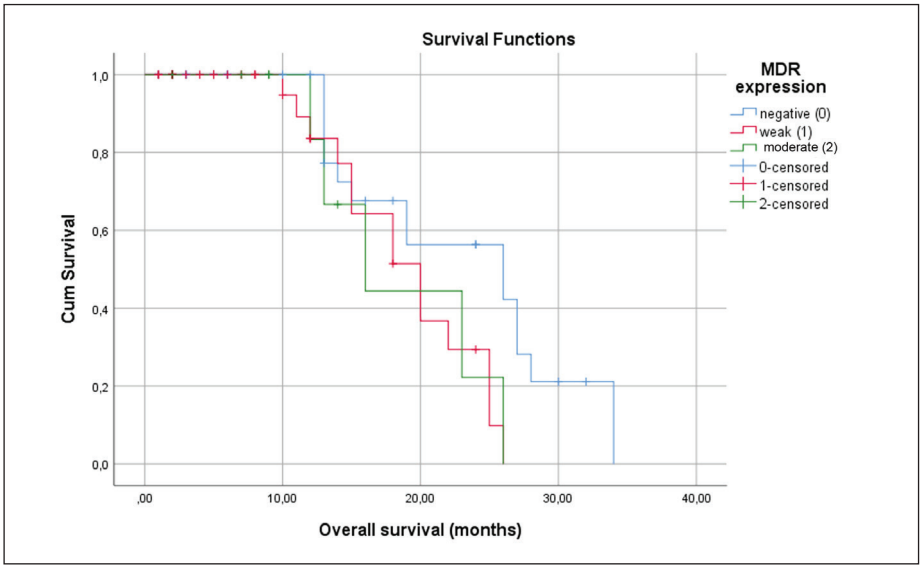

Figure 14: Overall survival of the patients with malignant ICT according to MDR1 expression patterns. Patients with negative expression of MDR1 had significantly better overall survival rate compared with those with positive expression both weak and strong (p=0.047).

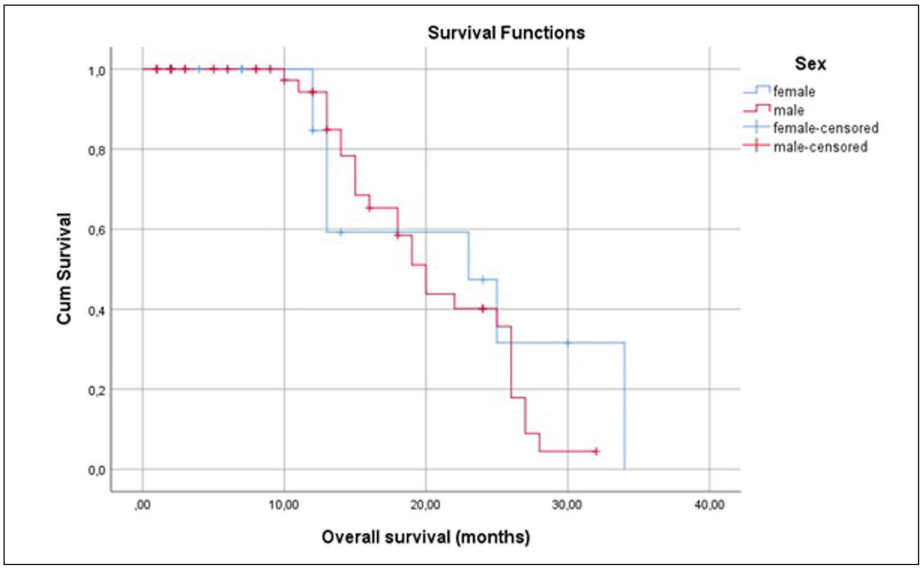

Figure 15: Overall survival analysis of patients with malignant ICT according to sex. Kaplan-Meier survival analysis showed that there is no significant association between sex and overall survival (p=0.545).

The GSTM, GSTP, CYP1A1, CYP1B1, and MDR expression assessment in different grades of glioma was performed. The highest MDR expression was detected in oligodendroglioma (grade 2) followed by GBM. MDR expression was not observed in diffuse astrocytoma and gliosarcoma. The tumor grade differences based on MDR expression were statistically significant ($p<0.05$) (Figure 16). However, no statistically significant difference was noted in GSTM, GSTP, CYP1A1, and CYP1B1 expressions between the tumor grades ($p>0.05$), which may be due to the limited number of tumor samples. Longer survival was seen in oligodendroglioma and anaplastic astrocytoma patients. However, the difference between overall survivals of the patients in terms of tumor grade was not statistically significant ($p>0.05$) (Figure 17).

DISCUSSION

In this prospective study, 141 patients were operated on for 149 intracranial lesions in a 27-month period. There were 142 tumor samples and 7 non-tumoral lesions. Among the tumor samples, 97 (91 patients) and 45 were malignant and were benign tumors, respectively. Among the 91 patients, 77 patients with malignant ICT diagnosis and complete medical records postoperative were investigated in detail for the relationship between the diagnosis, enzyme expression, and overall survival. Patients with negative expression of MDR1 had significantly better overall survival rate than those with positive expression both weak and moderate. No relationship was recorded between GST, CYP, and MDR1 expressions; alcohol consumption; and smoking. The most common tumors were metastasis and GBM.

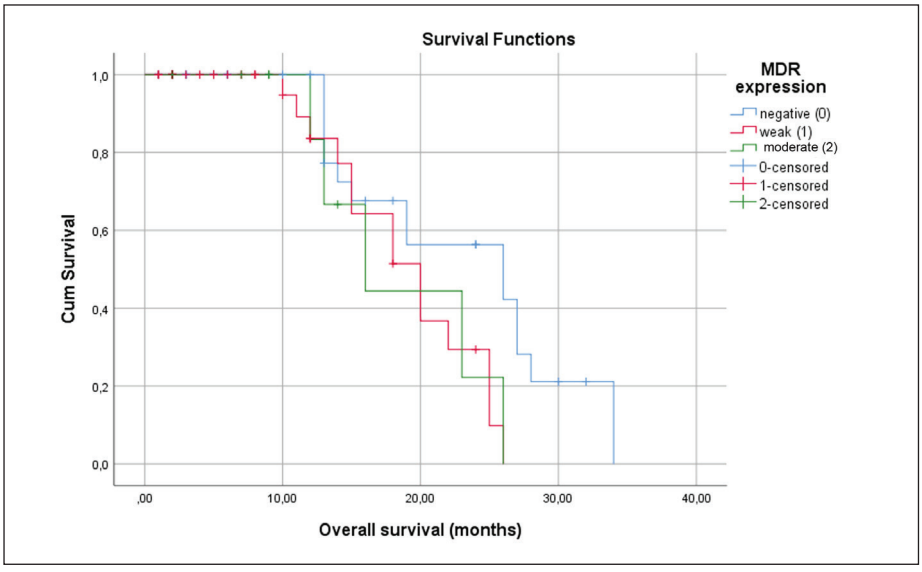

Figure 16: Staining intensity of GSTM, GSTP, CYP1A1, CYP1B1 and MDR in gliomas according to tumor grade. MDR expression was highest in patients with oligodendroglioma grade 2 and this was statistically significant ($p<0.05$). No statistically significant difference was found between GSTM, GSTP, CYP1A1 and CYP1B1 expressions and tumor grades ($p>0.05$).

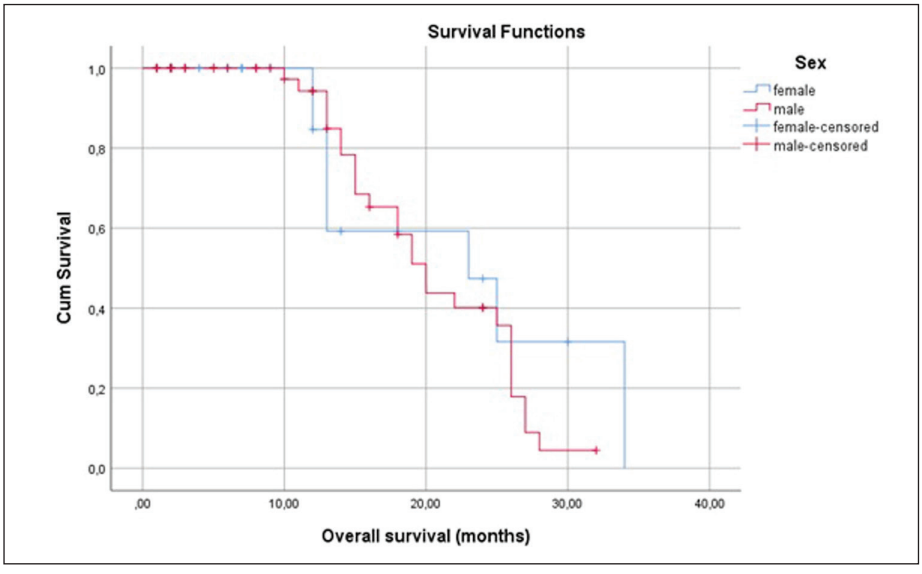

Figure 17: Bar diagram shows overall survival (months) of patients with malignant gliomas in terms of tumor grade. No statistically significant difference was found between overall survivals of the patients and tumor grade ($p>0.05$).

ICTs involve a large spectrum of tumors including the neoplasms of the brain or related structures (25). The most common ICTs are metastasis, glioma, and meningioma, which are classified based on their behavior as benign or malignant. Metastases are the secondary tumors of the brain originating from the extracranial tissues. A total of 20%–40% of all extracranial cancers will have one or more brain metastases (5,23). Malignant and benign intracranial neoplasms may increase intracranial pressure and cause neurological deterioration or death (8), making treatment a challenge. The histopathological feature of tumors is the main factor influencing the clinical presentation and patient prognosis (23). Today, intracranial metastases are more frequent and the most common malignant ICTs with gliomas (16).

Resistance to chemotherapeutic agents and some possible mechanisms for drug resistance (4) are substantial problems of the malignant ICT treatment. Increased expression of drug-metabolizing enzymes (GST and CYP), which contribute to chemotherapeutic agent inactivation in malignant neoplasms (1,21), may cause intracellular drug inactivation. Tumor microenvironment may also contribute to the progression of tumors and therapeutic resistance, especially in GBM (7). Oxidative stress may cause upregulation of the GST genes, which are overexpressed in many neoplasms, leading to disturbances through chemotherapy.

Human GSTs, which are located in the cytoplasmic matrix, can be grouped according to the similarity of sequencing and cross-reactivity in immunological features, having GSTM1, GSTT1, and GSTP1 as the most commonly referred ones (22,27). GSTs, primarily located in astrocytes, may have a neuroprotective function. In contrast, increased expression of GSTs possibly affects chemotherapy, which may occur by direct medicine degradation or by decreasing the medicine interaction with deoxyribonucleic acid and other intracellular materials (27). Many brain tumor reports have shown that susceptibility to intracranial tumors and the existence of GST variants are associated in adults' cases, in the last 20 years (9,17,24,26). In contrast, Lai et al. did not find any relationship between genetic pleomorphisms of GST enzymes and the development intracranial tumors in adult humans (20). Grant and Ironside found that GST immunostaining was apparent in astrocytes and endothelial cells but not neurons or oligodendrocytes in the human brain (10). Hara et al. reported diffuse weak GSTP expression in low-grade astrocytomas, similar to healthy glial cells. Higher-grade gliomas demonstrated a remarkable strong GSTP expression (13). Juillerat-Jeanneret et al. revealed that the intrinsic cell properties from GBM cell lines were heterogeneous expressions of MGMT and GSTP1, possibly making malignant tumors resistant to alkylating drugs (17). In our study, we used GSTP and GSTM in ICTs and found an increased expression of these proteins in brain tumors.

The most significant phase I medicine degradation enzymes are CYPs (28). A relationship between the expressions of CYPs and different neoplasms may exist. CYP1A1 plays an important role on the aryl hydrocarbon hydroxylase enzyme activation and in the functions of many carcinogenic agents (1,3) such as in the development of lung cancer (3,12). There are several studies on CYP1A1 polymorphisms and smoking-related cancers. Meanwhile, a close relationship between the CYP1A1 gene expression and enzyme activity was not revealed yet (3,12). The 4-hydroxylation of estrogens, which has an important role in hormonal carcinogenesis, is catalyzed by CYP1B1. Moreover, many environmental mutagens can be activated by CYP1B1 (12), which are overexpressed in the different types of neoplasms, such as breast, liver, prostate, and bladder cancers. The expression of CYP1B1 is absent or low in healthy tissues (12,14). Increased GSTP and CYP expressions have been previously shown in ICTs (13,19). In our series, we analyzed CYP1A1 and CYP1B1 expressions and found that these proteins were increased in ICTs. But there was no relationship between the GST and CYP expression and the survival of the patients with malignant ICT.

The ABC transporters, including P-glycoprotein (P-gp or MDR1) and multidrug resistance-associated protein 1 (MRP1), mediate the effect of hypoxia on resistance to chemotherapeutics. Increased expression of ABC transporters is one of the most prominent MDR systems, saving cancer cells from different chemotherapeutic agents (7), as shown in a few studies on the relationship between the MDR expression and brain tumors. Calatozzolo et al. show an association between multidrug resistance transporters and refractory epilepsy in glioma (6). Tivnan et al. showed that a considerable rise in vincristine- and etoposide-induced cell loss is caused by specific MRP1 inhibition in GBM cell lines. However, specific MRP1 knockdown did not affect the temozolomide response in GBM (29). Guimaraes et al. revealed that pomolic acid downmodulated the MRP1 activity and inhibition of GBM cell migration (11). In our study, we mainly focused on the survival of patients with malignant ICT and MDR1 expression and found the association between longer survival and negative MDR1 expression, which was not previously shown in clinical studies investigating the survival of patients with malignant ICTs.

CONCLUSION

Increased GSTP and CYP expressions were observed in ICTs. MDR1 is an important indicator of survival in patients with malignant intracranial tumor. Longer survival is associated with a negative MDR1 expression. Further studies with larger series and different enzyme subtypes are required for the confirmation of our results.

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