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Investigation of *Survivin* Gene Polymorphism and Serum Survivin Levels in Patients with Brain Tumors

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Abstract. *Background/Aim: The single nucleotide polymorphism -31C/G identified in the survivin gene promoter seems to be associated with over-expression of survivin, an anti-apoptotic protein. In gliomas, increased survivin expression correlated with decreased survival. The aim of the study was to investigate whether survivin gene polymorphism associates with benign and malignant brain tumors and whether it affects survivin serum levels. Patients and Methods: Survivin polymorphism -31C>G was genotyped in 82 patients with brain tumors and 65 healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and survivin levels were evaluated by enzyme-linked immuno sorbent assay (ELISA) in patients and controls. Results: Serum survivin levels in patients with malignant tumors were higher than patients with benign tumors (p<0.001). Survivin levels in patients with malignant glial tumors and the frequency of the GG genotype were higher than in patients with benign tumors (p=0.04) and controls (p=0.05). The prevelance of the survivin gene promoter polymorphism -31C>G did not differ between patients and controls. Conclusion: Survivin promoter -31C>G gene polymorphism seems to be associated with serum survivin levels in brain tumors of different grades and histologies.*

Molecular research findings have provided novel insights into the mechanisms of pathogenesis of brain tumors but their importance in predicting response to treatment and clinical progression is still unclear (1). Apoptosis is important both in embryogenesis and tissue homeostasis (2). Possible associations between expression of apoptotic markers such as bcl-2, bax, fas/fasL, survivin and p53 in brain tumors and disease prognosis have been investigated to identify prognostic markers (1). During fetal development, survivin, a member of the inhibitor of apoptosis gene family (IAP), is expressed to promote cell proliferation by inhibiting apoptosis, but in adult tissues it is scarcely detected (3). Survivin mRNA expression in adult brain is detected at a very low level when compared to survivin mRNA expression in normal fetal brain (4).

Meningiomas arising from meningoepithelial cells are mostly slow-growing benign lesions and patients are diagnosed usually during middle and old ages. There is evidence that deregulation of cell cycle has an important role in meningioma progression. Molecular mechanisms of formation, growth and malignant transformation of meningiomas are being investigated and newly discovered genetic changes have been associated with different tumor grades (5, 6).

Studies in recent decades have shown that primary and secondary glioblastomas may be associated with altered genetic and molecular pathways which involve activation and inactivation of several tumor supressor genes and oncogenes, MGMT promoter methylation status as well as gain and loss of heterozygosity such as 10q (7-9). Alterations in signaling pathways of *RTK/RAS/MAPK/PI3KA*; the p53 pathway - *TP53, p14ARF, MDM2, MDM4*; the RB pathway - *CDKN2A/CDKN2B, CDK4/CDK6, RB1* and changes in metabolic pathways involving mutations in isocitrate dehydrogenase-1 (*IDH1*) and *IDH2* are commonly reported involved in glioblastoma pathogenesis (10-13).

In primary glioblastomas, epidermal growth factor receptor (*EGFR*) amplification, *PTEN* mutations and $p16^{INK4a}$ deletions and absence of *IDH* mutations are more common,

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whereas early and frequent *TP53* mutations and IDH mutations are present in secondary glioblastomas (10, 12, 13).

The survivin gene (*BIRC5*) is located on chromosome 17q25, spanning approximately 14.7 kb and consists of 4 exons and 3 introns (3).

Survivin protein expression has been identified in most human cancers and high survivin expression has been found to be correlated with disease recurrence and decreased survival in malignancies including colorectal cancer (14), lung cancer (15) and renal cell carcinoma (16).

There is an inbalance between cell proliferation and apoptosis in carcinogenesis. Survivin protein is associated with both regulation of mitosis and inhibition of apoptosis. It has been suggested that by overexpression of survivin, the apoptotic check point is overcome and transformation of cells proceeds (17, 18). Survivin promoter single nucleotide -31G>C polymorphism is located on the CDE/CHR repressor binding region in the promoter of the gene (3, 17). The function of survivin in cell division and cell death networks has been investigated in order to determine its importance for cancer prognosis (18). It has been shown that changes in cell cycle dependent transcription in cancer cell lines and this polymorphism are associated with changes in both mRNA and protein levels (19). Survivin is frequently expressed in meningiomas and gliomas and it has been suggested that it is associated with tumor progression and poor prognosis (20). Several studies have examined the association of survivin gene promoter -31 C>G polymorphism with different types of cancer (21-24).

In this study, the association between *survivin* gene 31C>G polymorphism with brain tumors and the relationship between serum survivin levels and survivin polymorphism were investigated.

Patients and Methods

Patient selection and clinical investigation. A total of 82 patients with brain tumors were included in the current study. All cases were treated at the Neurosurgery Clinic of the Medical Faculty of the University within a period of 6 months. The diagnoses of the patients were determined by radiological and operative findings and confirmed by pathological examination. The blood samples were collected from the patients before any treatment (chemotherapy or radiotherapy). Malignancy status and histological origin were considered in grouping as arising from glial and meningothelial (nonglial) cells according to WHO classification of tumours of the central nervous system in 2007. Grade I tumors are benign and grades II, III and IV are considered as malignant (25).

The control group consisted of 65 healthy individuals who did not have any benign or malignant tumors and any family history of tumors.

The present study was approved by the Ethical Committee of Medical Faculty of the University. The informed consent was taken from all patients and healthy controls. The protocol followed was consistent with the Declaration of Helsinki.

DNA isolation. Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood as previously described (26).

Survivin gene -31C>G polymorphism genotypes were determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. A 341-bp PCR amplification product of survivin -31 C>G polymorphism was detected by using the forward 5'-GTT CTT TGAAAGCAGTCGAG-3'and reverse 5'-GCCAGTTCTTGAATGTAGAG -3' primers: The reaction mix contained, 50-100 ng genomic DNA, 1xpolymerase chain reaction (PCR) buffer, 0.2 mM of each dNTP, 3 mM MgCl2, 0.2 mM of each primer and 0.5 U of Taq polymerase (MBI Fermentas, Lithuania) in a 25 ml reaction volume. The PCR reactions were started with an initial denaturation of the DNA at 94˚C for 5 min, followed by 35 cycles of denaturation 94˚C for 30 sec, annealing at 57˚C for 90 sec and extension at 72˚C for 90 sec. The final elongation step was at 72˚C for 5 min. The PCR products were digested with EcoO109I restriction enzyme (MBI Fermentas, Lithuania) at 37˚C for 16 h followed by electrophoresis in 2% agarose gel containing ethidium bromide. The CC genotype lacks a EcoO109I site and shows only one band of 341 bp. The genotype GG generates two fragments of 236 and 105 bp. The heterozygote GC genotype displays three fragments of 341, 236 and 105 bp (24).

Serum survivin levels. The survivin serum levels were determined in blood samples by Enzyme-Linked Immuno Sorbent Assay (ELİSA) using Survivin EIA kit (human Total Survivin TiterZyme® EIA kit, Assay designs).

Statistical analysis. All statistical analyses were carried out using the SPSS version 7.5 statistical package for Windows. Student *t*-test or Mann–Whitney *U*-test, chi-square test, and Kruskal–Wallis tests were used to assess both the prevalence of the genotype of the *survivin* promoter region and allele frequencies and the levels of survivin protein between groups. Chi-square test or Fisher's exact test was also used to calculate Odds Ratio (OR) with 95% confidence intervals (95%CI) to determine whether survivin genotypes were associated with the disease. The threshold for significance was $p<0.05$.

Results

The patients' group involved 44.4% women and 55.6% men while the control group consisted of 45.5% women and 54.5% men. The mean age of the 82 patients was 61.57±14.34 years and the mean age of the 65 controls was 57.18±11.52. Those aged 60 years and less were 90.6% in the control group and 80% among patients ($p=0.164$). There were no significant differences between genders ($p=0.890$) and mean ages of patients and controls ($p=0.809$). None of the healthy individuals was smoking while 29.3% of the patients were smokers ($p=0.001$).

Only primary intracranial tumors were included in the study. The majority of the patients had gliomas (n=43; 52.4%) or meningiomas (n=31; 37.8%). The remaining patients had cranial nerve tumors (n=3; 3.7%), cellar region tumors (n=3; 3.7%), lymphoma and hematopoetic neoplasms (n=2; 2.4%).

Table I. *Survivin promoter -31 C>G genotype allele distribution in primary brain tumors classified according to the malignancy status and histology.*

Genotypes -31C >G	Malignant non-glial tumor patients n (%)	Malignant glial Tumor patients n (%)	Benign non-glial tumor patients n (%)	Benign glial tumor patients n (%)	Controls n (%)
CC	4 (36.4)	4 (10.5)	4 (14.3)	0 (0)	8 (12.3)
GC	5 (45.5)	21 (55.3)	14 (50)	3 (60)	26 (40.0)
GG	2 (18.2)	13 (34.2)	10 (35.7)	2 (40)	31 (47.7)
C allel	13 (59)[a]	29 (38.2)	22 (39.3)	3 (30)	42 (32.3)
G allel	9 (41)	47 (61.8)	34 (60.7)	7 (70)	88 (67.7)

[a]p=0.02; comparison of malignant non-glial tumor patients and control group.

Table II. *Survivin promoter -31C>G allele distribution according to characteristics of patients with meningeal tumors.*

Patient characteristics	Genotypes									
	CC+CG N (%)	GG N (%)	N	p-Value	OR 95%CI	GG+GC N (%)	CC N (%)	N	p-Value	OR 95%CI
Gender										
Men	12 (85.7)	2 (14.3)	14	0.068	1.619 (0.985-2.66)	11 (78.6)	3 (21.4)	14	0.698	1.113 (0.738-1.679)
Women	9 (52.9)	8 (47.1)	17			12 (70.6)	5 (29.4)	17		
Smoking status										
Smoker	7 (77.8)	2 (22.2)	9	0.677	1.222 (0.763-1.957)	6 (66.7)	3 (33.3)	9	0.66	0.863 (0.516-1.443)
Nonsmoker	14 (63.6)	8 (36.4)	22			17 (77.3)	5 (22.7)	22		
Age 1										
<45 years	7 (77.8)	2 (22.2)	9	0.675	1.256 (0.774-2.039)	4 (44.4)	5 (55.6)	9	0.03*	0.519 (0.245-1.099)
≥45 years	13 (61.9)	8 (38.1)	21			18 (85.7)	3 (14.3)	21		
Age 2										
<60 years	15 (62.5)	9 (37.5)	24	0.633	0.750 (0.467-1.204)	18 (75)	6 (25)	24	0.645	1.125 (0.611-2.073)
≥60 years	5 (83.3)	1 (16.7)	6			4 (66.7)	2 (33.3)	6		
Tumor grade										
III and IV	3 (100)	0	3	0.533	1.556 (1.180-2.050)	1 (33.3)	2 (66.7)	3	0.156	0.424 (0.085-2.127)
I and II	18 (64.3)	10 (35.7)	28			22 (78.6)	6 (21.4)	28		
Malignancy status										
Malign	8 (88.9)	1 (11.1)	9	0.205	1.504 (0.991-2.284)	5 (55.6)	4 (44.4)	9	0.185	0.679 (0.366-1.258)
Non-malign	13 (59.1)	9 (40.9)	22			18 (81.8)	4 (18.2)	22		
Frontal location										
No	20 (66.7)	10 (33.3)	30	1.00	0.667 (0.518-0.859)	22 (73.3)	8 (26.7)	30	1.00	0.733 (0.591-0.910)
Yes	1 (100)	0 (0)	1			0 (0)	1 (100)	1		

Patients with malignant tumors and benign tumors were 63.2% and 36.8% of patients, respectively. Yamada *et al*. have grouped tumors as glial malign, glial nonmalign, nonglial malignant and nonglial nonmalignant (27). So, we also grouped the tumors according to their malignancy level considering histology and behavior as malign-glial (51.3%), malign-nonglial (12%); benign non-glial (30.8%) and benign glial (6%) according to literature classification (25).

The GG, GC and CC genotype frequencies of *survivin* gene polymorphism -31 C>G for controls and patients are presented in Table I. There were no statistically significant differences in survivin -31 C>G genotypes and allele frequencies between the controls and the patients.

In meningeal tumor patients, there was no significant relationship between *survivin* gene promoter -31C>G polymorphism and patient characteristics including gender, smoking, tumor grade, and malignancy status. However G allele frequency was significantly lower in patients diagnosed at ages younger than 45 years while the CC genotype was higher in this group (Table II).

In glial tumor patients, there was no significant relationship between *survivin* gene promoter -31C>G polymorphism and patient characteristics including gender, age, tumor grade, and malignancy status. However, smokers carried the CC genotype at a significantly lower frequency than non-smokers (Table III).

Table III. *Survivin promoter -31C>G allele and tumor characteristics in patients with glial tumors.*

Patient characteristics	Genotypes									
	CC+CG N (%)	GG N (%)	N	*p*-Value	OR 95%CI	GG+GC N (%)	CC N (%)	N	*p*-Value	OR 95%CI
Gender										
Men	19 (73.1)	7 (26.9)	26	0.176	1.380 (0.833-2.288)	23 (88.5)	3 (11.5)	26	1.00	0.940 (0.783-1.128)
Women	9 (52.9)	8 (47.1)	17			16 (94.1)	1 (5.9)	17		
Smoking status										
Smoker	13 (76.5)	4 (23.5)	17	0.266	1.275 (0.842-1.929)	13 (76.5)	4 (23.5)	17	0.021*	0.765 (0.587-0.995)
Nonsmoker	15 (60)	10 (40)	25			25 (100)	0	25		
Age 1										
<45 years	12 (54.5)	10 (45.5)	22	0.081	0.682 (0.439-1.059)	20 (90.9)	2 (9.1)	22	1.00	1.010(0.829-1.230)
≥ 45years	16 (80)	4 (20)	20			18 (90)	2 (10)	20		
Age 2										
<60 years	22 (61.1)	14 (38.9)	36	0.083	0.611 (0.471-0.793)	33 (91.7)	3 (8.3)	36	0.474	1.100 (0.759-1.594)
≥60 years	6 (100)	0 (0)	6			1 (16.7)	5 (83.3)	6		
Tumor grade										
III and IV	22 (68.8)	10 (31.3)	32	0.473	1.260 (0.700-2.269)	28 (87.5)	4 (12.5)	32	0.558	0.875 (0.768-0.997)
I and II	6 (54.5)	4 (45.5)	11			11 (100)	0(0)	11		
Malignancy status										
Malign	25 (65.8)	13 (34.2)	38	1.00	1.096 (0.517-2.325)	34 (89.5)	4 (10.5)	38	1.00	0.895 (0.802-0.998)
Non-malignant	2 (40)	3 (60)	5			5 (100)	0 (0)	5		
Frontal location										
No	22 (78.6)	6 (21.4)	28	0.011	1.964 (1.026-3.760)	25 (89.3)	3 (10.7)	28	1.00	0.957 (0.794-1.153)
Yes	6 (40)	9 (60)	15			14 (93.3)	1 (6.7)	15		

Serum survivin levels and genotypes. Survivin serum levels in patients with primary brain tumors according to their histology are presented in Table IV. A significant difference in serum survivin levels (pg/ml) among patient groups ($p<0.001$) was found.

In patients with aggressive glioblastoma multiforme (GBM) and *survivin* (promoter -31C>G) CC, GC and GG genotypes, the serum survivin levels were 21.7, 20.9±4.7, 47.7±8.7, respectively. Patients with GG genotype had significantly higher serum survivin levels than controls carrying the same genotype (23.6±6.3), ($p=0.032$), (Table V).

Discussion

Survivin is thought to contribute to the formation and development of tumors and increased levels of survivin may be a negative prognostic factor for tumor growth (17). It is suggested that when survivin-microtubule interactions are disrupted in G_2/M phase, overexpression of survivin may favor apoptosis rather than acting as an inhibitor (28). This may explain the different results of survivin expression in various cancers.

Yamada *et al*. (27) grouped tumors as malign/benign and glioma/nonglioma and examined the relationship between survivin expression and histologic malignity in brain tumor samples. They reported that malignant tumors expressed survivin mRNA at significantly higher levels than benign tumors and gliomas also expressed higher levels of survivin mRNA than nongliomas (27).

Survivin promoter -31C>G gene polymorphism genotypes. In the present study, survivin promoter -31C>G gene polymorphism genotypes and allele frequencies between patients and controls were not different. In patients with tumors originating from meninges the frequency of the C allele was significantly higher than the control group. C allele carriers were significantly higher in malignant nonglial patients, than in the control group.

In addition, C allele frequency was found significantly higher in various cancers (22). Wang *et al*. found that C/G and C/C genotype carriers have increased risk of urothelial carcinoma compared to individuals with G/G genotype (21). It has been reported that in patients with lung cancer, G allele carriers have less risk of developing cancer than CC carriers and that G allele has significantly less promoter activity than C allele (29). Gazouli *et al*. reported that in patients with colorectal cancer, -31C allele and CC genotype frequency are significantly higher compared to healthy individuals and, furthermore, survivin mRNA levels in CC carriers were more than those in G allele carriers (22). In

Table IV. *Survivin serum levels in patients with primary brain tumors classified according to the malignancy status and histology (pg/ml).*

	Malignant non-glial tumors	Malignant glial tumors	Benign non-glial tumors	Benign glial tumors	Control group	*p*-Value*
Survivin Serum Levels (pg/ml)	17.14±7.18[a]	36.6±3.70[b,c,d]	13.11±3.95[e]	13.50±7.38	25.7±3.36	<0.001

[a]p=0.021; malignant glial tumor patients compared to malignant non-glial tumor patients; [b]p<0.001; malignant glial tumor patients compared to benign non-glial tumor patients; [c]p=0.039; malignant glial tumor patients compared to benign glial tumor patients; [d]p=0.024; malignant glial tumor patients compared to control group; [e]p=0.021; benign non-glial tumor patients compared to control group.

Table V. *Serum survivin levels (pg/ml) and Survivin Promoter -31C>G genotypes according to malignancy and histology.*

	Malignant non-glial tumors			Malignant glial tumors			Benign non-glial tumors			Benign glial tumors			Controls		
-31C>G Genotype	CC	GC	GG	CC	GC	GG	CC	GC	GG	CC	GC	GG	CC	GC	GG
Serum survivin levels (pg/ml)	18.4± 11.4	35.4± 34.4	13.4± 12.4	21.7	28.5± 6.2	41.6± 8.1[a,b]	4.1± 3.1	20.8± 8.4	12.9± 6.5[a]	-	10.7± 9.7	5.8	26.4± 13.2	30.6± 5.6	23.6± 6.3[b]

[a]p=0.041; malignant glial tumor patients compared to benign non-glial tumor patients; [b]p=0.059; malignant glial tumor patients compared to control group.

another study, association of gastric cancer risk and G allele carrier status was not detected, but GG and GC was found to be associated with distal gastric cancer and well-differentiated tumors (23). Cheng *et al*. found that C allele and CC genotype frequency in *survivin* gene promoter -31C>G polymorphism were significantly higher in patients with stomach cancer than healthy controls but there was no association between genotypes and survivin mRNA (30). In breast cancer patients, the prevalence of genotype GC+CC was significantly higher compared with the control group (31). However, Borbely *et al*., reported that *survivin* gene promoter -31C>G polymorphisms were not associated with increased risk in cervical cancer development (24).

According to our results, in meningeal tumor patients, G allele frequency was significantly lower in patients diagnosed at ages younger than 45 years, while the CC genotype was higher in this group. The C allele may be associated with a young onset of meningeal origin tumors, whereas the G allele may be associated with young onset of malignant glial tumors. In GBM patients under 45, the GG genotype was significantly higher than in patients over 45.

It has been reported that the mean age of patients with secondary glioblastomas was 45 years (10). *De novo* and secondary gliomas affect patients at different ages through different pathways (7). There may be different molecular mechanisms in GBM development. Advanced patient age is a negative prognostic factor. Primary glioblastomas tend to develop in older patients (mean, 50-55 years), whereas patients with secondary glioblastoma are typically diagnosed at around 40 years of age (10, 32).

Serum survivin levels and survivin genotypes. Survivin expression increases with the degree of malignity of cancers. In colorectal cancer, survivin expression increased in adenoma, low grade dysplasia (2.3%), high grade dysplasia (52.4%) and carcinoma (63.3%) (14). In pancreatic ductal adenocarcinomas, the highest expression is found in ductal carcinoma while there is not any expression in normal pancreatic canals (33). Survivin expression has been found to increase as malignity increases, by 21.2% in benign tumors, 47.8% in borderline tumors and 51.2% in carcinomas of ovaries (34).

In human malignities, increased survivin expression may be associated with tumor aggressiveness and poor prognosis. It has been suggested that altered binding to the CDE/CHR repressor motive of the *survivin* gene promoter -31C>G polymorphism changes cell cycle transcription and thus both survivin mRNA and survivin protein level are effected (19).

In our study, serum survivin levels of patients with tumors originating from glial cells were found to be significantly higher than survivin levels of patients with tumors originating from non-glial cells and the latter had significantly lower levels than the controls. Thus, in our

patients with malignant nonglial tumors, serum survivin levels were significantly lower than the patients with malignant glial tumors.

Survivin levels have been examined in body fluids such as urine of patients with bladder cancer for diagnostic and prognostic reasons (21, 35).

Survivin levels in serum and urine of node-positive breast cancer patients patients were significantly higher than serum levels in node negative patients (36). Following chemotherapy of patients with locally advanced gastric cancer, serum survivin levels were significantly higher than controls and this may be used for the prediction of response to chemotherapy (37).

In another study, patients with nonsmall cell lung cancer and controls had similar survivin serum levels, but patients who responded to chemotherapy had decreasing levels of survivin (38).

High cytoplasmic survivin levels were reported to be associated with glioblastoma while high p53 expression and high nuclear survivin expression were associated with anaplastic astrocytoma (39). Furthermore, it was shown that the presence of high p53 levels or cytoplasmic or nuclear survivin in diffuse astrocytic tumors were associated with better prognosis following radiotherapy (39). It was also reported that in different grades of gliomas survivin may have different effects on prognosis (40).

In our study, the serum survivin levels in patients with malignant glial tumors with GG genotype were significantly higher than in patients with benign nonglial tumors and also controls having the same genotype. Patients with advanced neuroepithelial tumors carrying the GG genotype had higher survivin levels. Chakravarti *et al*., reported that patients with higher grade tumors (III.-IV.) had higher levels of survivin in sera than those patients with more benign tumors (I.-II. grade) and increase in survivin expression significantly correlated with decreased survival times in gliomas (40).

Uematsu *et al*. concluded that high nuclear survivin expression may be a negative prognostic factor in gliomas, while Preusser *et al*. reported that nuclear expression of survivin in GBM patients did not have an effect on prognosis (41, 42).

In a recent meta-analysis of 16 studies, it was concluded that prognosis in patients with gliomas was associated with high expression of survivin (43). Whether survivin is expressed in the nucleus or cytoplasm has different impact on prognosis according to recent studies (41-44). The differences in the results of the studies regarding expression and quantity may be due to the lack of a standardized procedure involving precise cut-off values for expression of survivin (45).

This study was performed before WHO 2016 guidelines, which recommends molecular markers to be involved in the identification of tumors of the central nervous system, were established (46). Our findings are similar to studies involving survivin mRNA and survivin protein level.

Strengths and limitations of the study. Number of patients may seem as a limitation at first, but it should be considered that we have chosen our group of patients according to very strict criteria, which is one of the strengths of this study and patient numbers are similar to studies involving brain tumors. All patients had pathologically-proven primary brain tumors after brain surgery and patients with metastatic tumors in brain were excluded. The patients should be followed in order to obtain precise results about prognosis and genotype relationship and the study must be verified in larger patient groups.

Conclusion

Survivin gene promoter -31C>G polymorphism seems to be associated with serum survivin levels in brain tumors with different grades and histologies. Malignant glial tumor patients with GG genotype, had higher survivin levels than benign non-glial tumor patients and controls having the same genotype. The findings of our study may have a clinical value in evaluating the prognosis and genetic risks of brain tumors besides providing a view into the underlying mechanisms of brain tumors.

Conflicts of Interest

The Authors declare no conflicts of interest.

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