in vivo *32*: 287-290 (2018)
doi:10.21873/invivo.11236

Tumor Necrosis Factor-Alpha (*TNF-α*-308 G>A) Polymorphism in High-grade Gliomas

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Abstract. *Background/Aim: High-grade gliomas (HGG) consist of anaplastic oligoastrocytomas, anaplastic oligodendrogliomas, anaplastic astrocytomas and glioblastoma multiforme. The present study aimed to evaluate TNF-α -308 G>A polymorphism in a Turkish population. Patients and Methods: This was a prospective case-control study that included 45 patients with HGG and 49 healthy individuals. All patients were operated for intracranial tumors and the pathology results consist of high grade (Grade3 and 4) glial tumors. Results: No significant differences were found between the HGG and control groups in terms of the median age (p=0.898). There were no significant differences with regard to gender (p=0.577). The TNF genotype frequency comparison between patients and controls was not statistically significant (p=0.598). Conclusion: TNF genotype frequency comparison between the patients and controls was not statistically significant in the Turkish population tested. However, further studies are needed to evaluate the genotype and phenotype correlations in large cohorts of various ethnicities.*

High-grade gliomas (HGG) consist of anaplastic oligoastrocytomas, anaplastic oligodendrogliomas, anaplastic astrocytomas and glioblastoma multiforme (GBM) (1). GBM and anaplastic astrocytomas consist the 80% of primary central nervous system gliomas (2). The molecular genotype is the new consideration of subclassifying these tumors by the World Health Organization (3). Maximal safe resection is the gold standard treatment for newly diagnosed HGG which must be followed by radiation therapy and chemotherapy.

Impaired immune reaction or defective oncosuppressive mechanisms in tumor cells are related to tumor growth process (4). Th2 type lymphocytes which are connected with the deficit of im-mune response, took part in infiltrating process of glioma (5). Th1 cytokines which increase cellular immunity also inhibit Th2 cells. Alterations in the production of Th1 cytokines may be connected with immunological response to prevent tumor formation (6). These results motivated us to evaluate the frequency of polymorphisms of the tumor necrosis factor (TNF) region.

The polymorphisms that affect the production of TNF-α have been reported as causes of some diseases (7). Here, we evaluated the frequency of *TNF-α*-308 G>A polymorphism in a population of Turkish HGG patients and a healthy control group. Our study is the first research examining *TNF-α*-308 G>A polymorphism in HGG.

Materials and Methods

Study population. This was a prospective case-control study that included 45 patients with HGG. All of the participants were selected from the Neurosurgery Departments of Yeditepe University and Cerrahpaşa University in Istanbul, Turkey. All patients were operated for intracranial tumors and the pathology results consist of high grade (Grade-3 and 4) glial tumors. A total of 49 healthy individuals were selected for the control group. Informed consent forms were obtained from all of the patients.

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Key Words: Tumor necrosis factor, polymorphism, high-grade gliomas.

Genetic analysis. The peripheral blood samples were collected into EDTA tubes. The DNA extraction was performed using an iPrep Purification Instrument (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA) with 350 μl of peripheral blood and an iPrep PureLink gDNA blood isolation kit (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's instructions. The isolated DNA samples concentrations were then measured with a NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, MA, USA) using a 1.7-1.9 optical density range for the genotyping and final sample concentrations were diluted to approximately 100 ng/μl. The *TNF-α*-308 G>A (rs1800629) polymorphism genotyping was performed using the 7500 Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) with the TaqMan Genotyping Assay, TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA), and 100 ng of sample DNA. The reaction mixture and conditions were used as recommended by the manufacturer: 10 min at 95˚C for the holding stage, 40 cycles of 15 sec each at 92˚C for the denaturation, and 60 sec at 60˚C for the annealing/extension. The allelic discrimination of the samples was done by collecting and interpreting the fluorescent signals of the hybridization probes using the software from the 7500 Fast Real-Time PCR instrument.

Statistical analysis. The statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). The significance of differences between the groups were examined with the Student's *t*-test, and the comparisons of the demographic information were evaluated with the chi squared and Fisher's exact tests. The statistical significance level was $p<0.05$.

Table I. *Demographic characteristics of patient and control groups.*

Parameter	Patient (n=45)	Control (n=49)	*p*-Value
Age Mean±SD	34.62	34.81	0.898
Gender Male/Female	20/25	19/30	0.577

Table II. *Genotype and allele frequencies between patient and control groups.*

Genotype rs1800629	Patient (n=45)	Control (n=49)	*p*-Value
GG	41 (91.9%)	43 (87.8%)	0.598
GA	4 (8.9%)	6 (12.2%)	0.598
AA	0 (0%)	0 (0%)	-
Allele		Allelic Count	
G	86 (95.5%)	92 (93.8%)	-
A	4 (4.5%)	6 (6.2%)	0.598

Results

Our analysis included 45 HGG patients and 49 healthy individuals as a control group. The demographic characteristics of the two groups are shown in Table I. The mean ages of the patients with HGG and the healthy controls were 34.62±8.26 years old and 34.81±4.53 years old, respectively. No significant differences were found between the HGG and control groups in terms of the median age (p=0.898). The gender frequency was 44.4% male and 55.6% female for the patients and 38.8% male and 61.2% female for the controls. There were no significant differences with regard to gender (p=0.577).

The allele and genotype frequencies of *TNF-α*-308 G>A polymorphism in the patients with HGG and the control group are shown in Table II. The TNF genotype frequency comparison between the patients and controls was not statistically significant (p=0.598). There was no AA genotype detected neither in patient nor in the control group.

Discussion

HGG is one of the most invasive and aggressive cancers that is fatal although all treatment modalities like surgery, systemic chemotherapy and radiation are nowadays applied (8). As this disease is characterized by aggressive local invasion but not distant metastasis, local delivery of radiation in the form of SRS has been and continues to be attempted as a treatment strategy in combination with other treatment modalities with variable reported success rates (1). Without any treatment, patients with recurrent HGG have a median survival of about 3 to 6 months (9).

Clinical presentations of HGG are progressive neurologic signs and symptoms that vary according to the location and size of the tumor. Symptoms could be counted as seizure, headache, motor weakness, memory loss, cognitive problems, personality changes, visual symptoms (10). Prognostic factors of HGG patients are age, Karnofsky performance score, tumor grade, the number of molecular alterations and ratio of surgical resection (11). Recently, biomarkers like methylation of methyl guanine methyl transferase (MGMT) as a gene expression analysis are being used for prognostication (12). In elderly patients with HGG, MGMT predicts better survival rate (13, 14). MGMT enzyme promotes DNA-repair following alkylating agent chemotherapy and loss of chromosomes 1p and 19q improves responsiveness to chemotherapy and survival in oligodendroglial tumors (15). Isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* enzyme mutations also improves overall survival independent of other prognostic factors especially in HGG (16).

Impaired immune reaction or defective oncosuppressive mechanisms in tumor cells are related to tumor growth process (4). Th2 type lymphocytes that are connected with the deficit of immune response, took part in infiltrating process of glioma (6). Pociot *et al*. (1993) found a correlation between TNF microsatellite alleles and *in vitro* lipopolysaccharide-stimulated TNFa production (7). The regulation of the production of TNFa and lymphotoxin (TNFb) is not known clearly, but a relation to human leukocyte antigen alleles was suggested (17). TNF production could be related to the development of different

types of immune response. For example, decreasing the amount of TNF producers could develop a type-2 (Th2) response which would result as reduction in antitumor activity (6). In the literature, Th2 type lymphocytes which are connected with the deficit of immune response, have been reported as taking part in the infiltrating process of glioma (5).

Only a few studies reported TNF polymorphisms in gliomas in the literature. Frigerio *et al*. (1999) reported in their microsatellite polymorphism study that *TNFb4*-negative individuals could preferentially develop a Th2-type immune response which could result as a reduction in antitumor activity in Italian GBM patients (4). Crouau-Roy *et al*. (1993) also investigated TNF microsatellites in European population but not in gliomas (18). Our study is the first research regarding the *TNF-α*-308 G>A (rs1800629) polymorphism in HGG, but we found that *TNF* genotype frequency comparison between the patients and controls was not statistically significant in the Turkish population.

Conclusion

This study evaluated the relevance of the *TNF-α*-308 G>A polymorphism with regard to the risk of HGG. We found that *TNF-α*-308 G>A genotype frequency comparison between patients and controls was not statistically significant in the Turkish population studied. Since genetic polymorphisms often show ethnic differences, further functional studies are needed to evaluate the genotype and phenotype correlations in large cohorts of various ethnicities.

Conflicts of Interest

The Authors declare no conflicts of interest in regard to this study.

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Received November 28, 2017
Revised December 19, 2017
Accepted December 21, 2017