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# Serum Levels of BDNF, TNF alpha, Caspaz-3, AChE, BChE in Patients with Brain Tumor

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## ABSTRACT

**Objective:** Brain tumor development mechanisms are still the subject of current research, and treatment models are improving. Our study aimed to investigate the association of cytokines with intracranial tumor types and grades and their usability in diagnosis and follow-up.

**Material and Methods:** This study included 48 individuals (24 brain tumors and 24 control groups). The blood samples were taken from the study groups, and biochemical analysis was performed. The groups were then compared for statistical significance.

**Results:** In our study, our aim was to investigate the relationship between enzyme levels and intracranial tumors in various pathologies and grades. We found a significant association between TNF-α, Caspase-3, and BChE enzymes, but this association was not observed for BDNF and AChE.

**Conclusion:** We measured the levels of various cytokines secreted in gliomas and other brain tumors and carried out our study to determine whether these measurements cause changes in the staging of the disease, their use as a diagnosis, and the length of stay. This study would shed light on more comprehensive studies on brain tumors.

**Keywords:** Acetylcholinesterase, Brain tumor, Butyrylcholinesterase

## INTRODUCTION

Approximately 70% of primary intracranial tumors are centrally located. While it commonly originates from the nervous system, 30% originates from the meninges (1). Tumor development mechanisms are still the subject of current research, and treatment models are improving daily. An essential part of these treatment models is related to the immunomodulatory function.

The brain tumor microenvironment consists of neoplastic and non-neoplastic cells. Non-neoplastic cells include macrophages, astrocytes, endothelial cells, microglia, and lymphocyte cells. Among non-neoplastic cells, microglial cells and macrophages make up 30-50% of the total brain tumor mass. The relationship between neoplastic and non-neoplastic cells plays a critical role in invasion and tumorigenesis (5, 6). Brain tumors may contribute to the development of drug resistance by forming a complex microenvironment, the mechanism of which has not yet been fully resolved. Recently, some drugs that neutralize neoplastic cells by affecting brain tumor progression have been used (2-4).

Microglia are the resident macrophage of the CNS. It plays an active role in host defense and immune surveillance against infectious agents and neoplastic tumors. Under physiological conditions, microglia, characterized by branched morphology, are at rest (7). Traumatic and infectious stimuli cause many changes in microglia morphology. Microglia rapidly revert to the "amoeboid" phenotype, producing reactive oxygen species and nitric oxide, altering gene expression.

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In addition, proinflammatory chemokines and cytokines control the clearance of pathogens. Although these effects seem positive in terms of defense mechanism at the first stage; prolonged and chronic microglial activation can cause pathological forms of inflammation that contribute to the formation of neoplastic and neurodegenerative diseases (8). Although activated microglia are believed to secrete cytotoxic factors that suppress or destroy cancer cells and pathogens, they can also produce growth factors that increase cell survival, growth, and neuron function (9,10). Our study aimed to investigate the changes in cytokine levels according to the type and grade of intracranial tumors, and to evaluate their potential use in diagnosis and monitoring of these tumors.

## MATERIAL and METHODs

In the study, blood samples were drawn into heparinized tubes from 48 individuals (24 patients and 24 control groups). The blood samples were centrifuged at 3500 rpm for 10 minutes, and the upper plasma was separated for the study. The collected plasma samples were frozen at -18 °C until the analysis was performed. The image and intraoperative film of the high-grade glial patient with the brain tumor is shown in **Figure 1**. The local ethics committee approved this study (protocol no: 2022/13).

### AChE/ BChE Enzyme Activity

AChE and BChE enzymes were determined spectrophotometrically according to the Ellman method. The Ellman method uses thiol ester acetylthiocholine instead of oxy ester acetylcholine as a substrate. According to the Ellman method, acetylthiocholine is hydrolyzed by acetylcholinesterase, and the thiocholine released as a result of hydrolysis reacts with the Ellman reagent DTNB [5,5'-dithio-bis-(2-nitrobenzoic acid)]. As a result of the reaction, yellow-colored chromophore TNB (5-thio-2-nitrobenzoic acid) is formed. The rate of formation (color intensity) of this yellow compound formed at the end of the reaction is determined by measuring the absorbance at 412 nm. (Ellman, Courtney, et al.) The intensity of this yellow color is directly proportional to the AChE/ BChE enzyme activity.

### Apoptosis (Caspase-3) Determining the Level

Caspase-3 enzyme activity was determined in the control and experimental group samples using the “Caspase-3 Analysis Kit” (Fish (CASP3) ELISA Kit (Catalog No: 201-00-0031) (SunRed)). The main objective of this analysis is to identify the product generated as a result of the reaction between the substrate and caspase-3 enzyme. Readings were done on 10-minute ELISA (plate reader) instruments at 450 nm absorbance.

### BDNF (Brain-derived neurotrophic factor)

Serum Brain-derived neurotrophic factor (BDNF) levels were determined using the ELISA kit. The standards prepared by bringing the kit materials to room temperature half an hour before starting the work are added to the Microelisa Strip Plate. Then, the necessary Kit procedures were applied, and measurements were made within 10 minutes in an ELISA (Plate Reader) device with 450 nm absorbance.

### TNF-α levels

TNF-α levels in samples were measured by enzyme-linked immunosorbent assay (ELISA) uh commercial kits (Human HIF-1α ELISA kit, Human VEGF ELISA kit, Human TNF-α ELISA kit, Sunred Biotechnology, Shanghai, China) following the manufacturer's instructions.

### Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 4.0 software (GraphPad Software, 2003, San Diego, CA, USA). All data are presented as mean ± standard error). Groups were compared mann-whitney u test. Groups of data were compared with an analysis of variance (ANOVA). Values of $p<0.05$ were considered significant.

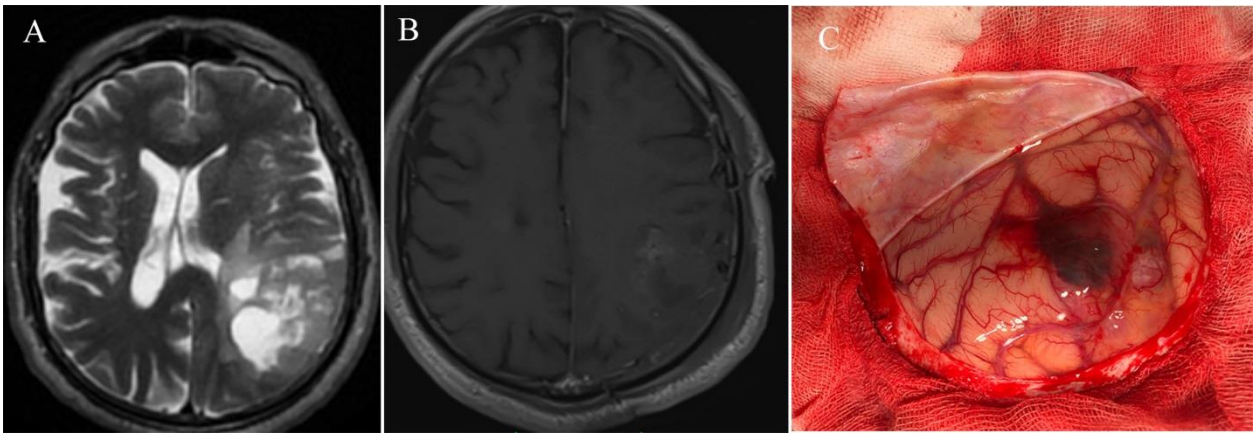


**Figure 1.** Intraoperative image of the high-grade glial brain tumor.

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## RESULTs

The mean TNF-α values of the patient group were significantly higher than controls (p<0.001) (Figure 2). Although the difference between the control and patient groups in terms of mean BDNF levels was statistically insignificant, the mean BDNF values of the patient group were higher (p>0.05) (**Figure 2**).

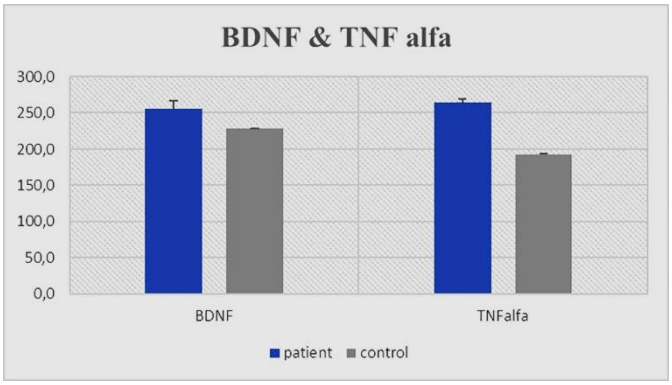


**Figure 2.** The mean TNF-α and BDNF values of the patient and control groups.

The mean Caspase-3 values of the patient group were higher than controls (p<0.001) (Figure 3). Although the difference between the control and patient groups in terms of mean AChE levels was statistically insignificant, the mean AChE values of the patient group were higher (p>0.05) (Figure 3). The difference between the control and patient groups in terms of mean BChE levels was found to be statistically significant, and the mean BChE values of the patient group were higher (p<0.001) (**Figure 3**).

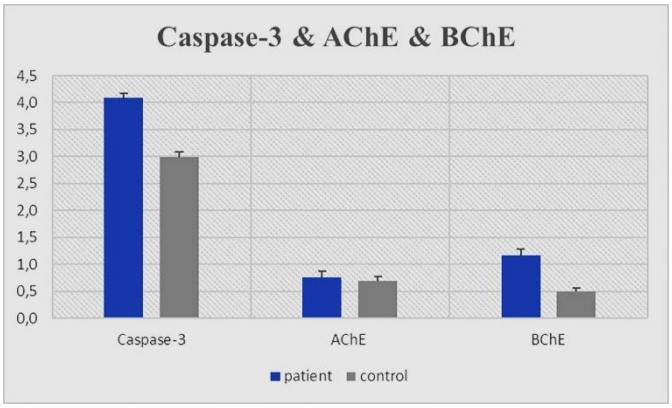


**Figure 3.** The mean Caspase-3, AChE, and BChE enzyme levels of the groups.

When the mean TNF-α, BDNF, Caspase-3, AChE, and BChE enzymes in the patient group were analyzed in terms of male and female genders, no statistical significance was found (p>0.05). When the mean TNF-α, BDNF, Caspase-3, AChE, and BChE enzymes in the patient group were analyzed in terms of smokers (n=7) and non-smokers (n=17), no statistical significance was found (p>0.05).

When the results of the TNF-α enzyme were examined in terms of the number of days hospitalized in the patient group, a statistically significant difference was found between those who had 8-14 (251.88±4.9) and 15-21 (288.92±11.3; p<0.05) hospitalization days (**Figure 4**).

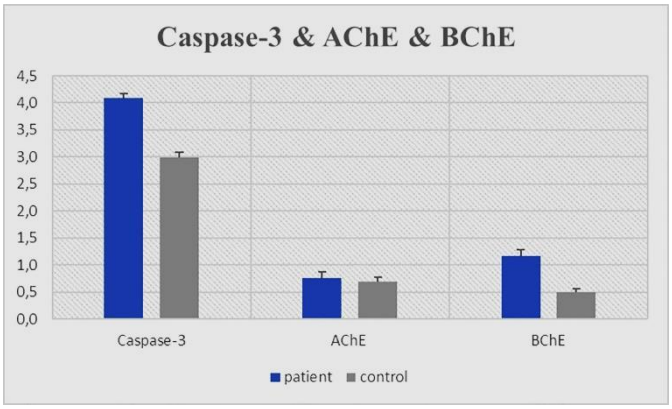


**Figure 4.** The mean TNF-α levels and hospitalization days.

When the averages of AChE enzyme were examined in terms of the number of days hospitalized in the patient group, a statistically significant difference was found between those who had 8-14 (1.05±0.19) and 15-21 (0.35±0.19; p<0.05) hospitalization days (**Figure 5**).

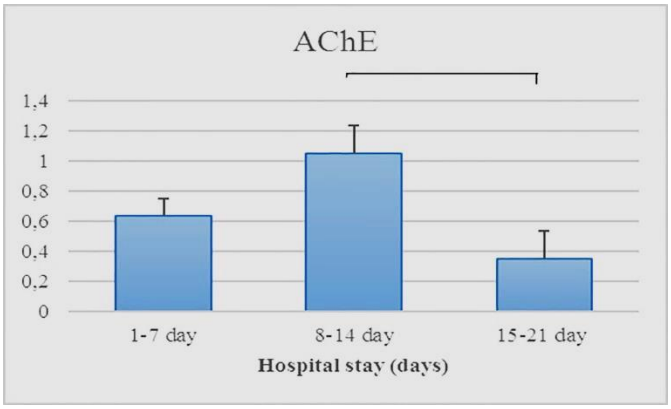


**Figure 5.** The mean AChE levels and hospitalization days.

When we compared the mean levels of TNF-α, BDNF, Caspase-3, AChE, and BChE enzymes among different tumor pathologies in the patient group, there was no statistically significant difference found (p>0.05) for meningioma, epidermoid cyst, hemangioblastoma, and glial tumors. When the mean TNF-α, BDNF, Caspase-3, and BChE enzymes in the patient group were examined in terms of tumor grade, there was no statistical significance (p>0.05). When the averages of AChE enzyme were compared, tumor grade 1 (0.73±0.09; n=17); was found statistically significant for patients with tumor grade 2 (1.64±0.38; n=3) and tumor grade 4 (0.17±0.04; n=4; p<0.05) (**Figure 6**).

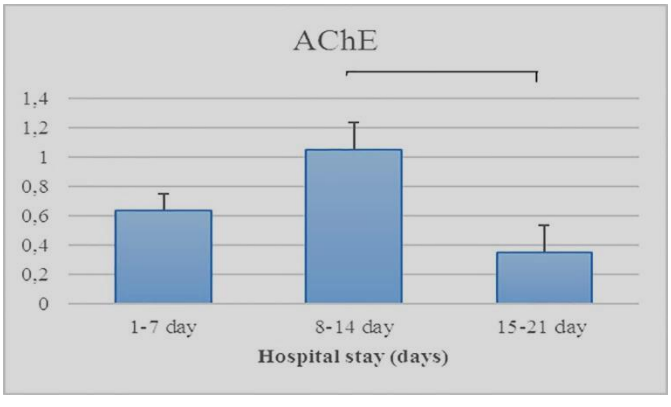


**Figure 6.** AChE enzyme levels and tumor grade.

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**Table 1.** CT enzyme study 24 patients.

| | | Patient | Control |
|---|---|---|---|
| **Age (mean ± SE)** | | **40.17± 3.1** | **41.02± 1.2** |
| **Gender** | **Female** | **15** | **13** |
| | **Male** | **9** | **11** |
| **Tumor pathology** | **Menengiom** | **12** | |
| | **Epidermal cyst** | **5** | |
| | **Hemangioblastoma** | **4** | |
| | **Glial** | **3** | |

**Table 2.** Comparison of enzyme levels between patient and control groups.

| Enzymes | Groups | N | average | Std. hata | T-test | |
|---|---|---|---|---|---|---|
| | | | | | *t* | *p* |
| **TNF-alfa** | **Patient** | **24** | **264.06** | **5.28** | **7.55** | **0.0001** |
| | **Control** | **24** | **192.28** | **7.89** | | |
| **BDNF** | **Patient** | **24** | **255.04** | **11.87** | **1.75** | **0.08** |
| | **Control** | **24** | **227.82** | **10.01** | | |
| **Caspaz-3** | **Patient** | **24** | **4.09** | **0.07** | **8.65** | **0.0001** |
| | **Control** | **24** | **2.98** | **0.1** | | |
| **AChE** | **Patient** | **24** | **0.75** | **0.11** | **0.41** | **0.68** |
| | **Control** | **20** | **0.69** | **0.07** | | |
| **BChE** | **Patient** | **24** | **1.16** | **0.12** | **4.55** | **0.0001** |
| | **Control** | **20** | **0.49** | **0.06** | | |

# DISCUSSION

It is essential to understand the communication of the tumor with the surrounding tissues, cytokines, and other molecular pathways in tumor development and treatment. The tumoral tissue uses various cytokines to avoid immune response and obtain support from the surrounding tissues for tumor growth. Gliomas are often referred to as "cold tumors" in the literature due to their ability to modulate the immune system, leading to a high level of T-cell suppression within the tumor microenvironment (11). Despite advances in immunotherapy treatment models, overall survival in patients with glioma has remained relatively high in the modern era. This is due to the ability of gliomas to suppress local and systemic immune responses, severely limiting the efficacy of treatment (12).

We measured the levels of various cytokines secreted in gliomas and other brain tumors and carried out our study to determine whether these measurements cause changes in the staging of the disease, their use as a diagnosis, and the length of stay. The difference between the control and patient groups in terms of mean TNF-α levels was found to be statistically significant, and the mean TNF-α values of the patient group were higher. In a study conducted by Qingxia Wei et al. in 2021, they found that glioma cells induce TNF-α secretion from macrophages through immunomodulation, which promotes neovascularization (13). Other studies found the preoperative TNF-α value to be high in gliomas (14). There was no significant difference between tumor type and grading and TNF-α level. Similarly, no significant difference was found in a publication looking at TNF-α levels in glioma grading (14). Although studies show TNF-α levels in glioblastoma cells, no statistical difference was found between the control and patient groups in terms of mean BDNF levels in our study (15).

When the patient group and control group were compared in terms of mean caspase-3 levels, caspase-3 levels in the patient group were statistically higher than in the control group. It was reported in a study that the proapoptotic caspase-3 story was found to be high, especially in patients with high turnover, especially with glioma (16).

There was no statistical difference between the control and patient groups regarding mean AChE levels. A significant difference was found in the BChE level. Studies show that AChE and BChE levels are found to be high in gliomas in various imaging techniques (17). In a study in which antioxidants were measured in patients with benign and malignant brain tumors, it was shown in the literature that AChE levels were significantly lower in patients with malignant brain tumors than in patients with benign brain tumors (18). However, no such significant difference was found in our research. Our study found that AChE decreased significantly as the malignancy increased from grade II tumors. This is our finding, Obukhova et al. It has also been shown in a similar study (19).

# CONCLUSION

In our study, we tried to reveal the relationship between enzyme levels and intracranial tumors in various pathologies and their grades. Significant association with TNF-α, Caspase-3, and BChE enzymes emerged. However, this association was not valid for BDNF and AChE. While the majority of the studies were on glial tumors, the number of glial tumors in our study was low. While shedding light on other tumor types is one of the positive aspects of our research, it would be beneficial to re-evaluate these findings in a more extensive series in terms of numbers.

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**Author Contributions: AT, AA, GG, ACY, SAE, NA**: Conception and design of the study, Analyzed the data, **AT**: Manuscript preparation, Revisions. All the authors have read and confirm that they meet, ICMJE criteria for authorship.

**Ethical approval:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study. Written consent was obtained from each patient to use their hospital data.

**Abbreviations:** CNS- central nervous system; BDNF- brain-derived neurotrophic factor; TNF-α- tumor necrosis factor alfa; AChE- acetylcholinesterase; BChE- butyrylcholinesterase; DTNB- 5,5'-dithio-bis-2-mtrobenzoic acid; TNB- 5-thio-2-nitrobenzoic acid.

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