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Investigation of BDNF, apoptosis and cholinergic system enzymes in glioblastoma multiforme patient serum

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ABSTRACT

Background: Glioblastoma Multiforme (GBM) is the most common type of brain tumor and it is an aggressive form of adult brain cancer that is globally fatal. Unfortunately, the poor prognosis of patients with glioblastoma and current treatments do not appear to be very effective against glioblastoma as the disease is still fatal. Our study aimed to explore new ways of diagnosing the disease to develop a treatment for this deadly disease.

Method: This study included 40 individuals (20 glioblastoma multiforme 20 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: Our study aimed to determine the relationship between enzymes, some parameters levels and glioblastoma multiforme. When compared to the control group, the levels of GSH-Px, SOD, CAT, AChE, and BChE were decreased in patients with GBM, and the levels of MDA caspase-3, BDNF, TNF alfa, and 8-OHdG were increased.

Conclusion: The results noted here point out that Apoptosis, Cholinergic System Enzymes, and BDNF are good and accurate markers in Glioblastoma Multiforme to know of diagnosing the disease. Will make it possible to use characteristics seen in glioblastoma multiforme as indicators for assessing tumor aggressiveness, as well as to get a better understanding of Proteins that are involved in apoptosis as well as anti-apoptotic proteins, allowing for the creation of novel molecularly based treatments.

Keywords: antioxidant enzymes, brain tumor, glioblastoma multiforme, cholinergic system enzymes

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ABBREVIATIONS

8-OHdG- 8-hydroxy 2-deoxy guanosine
AChE- Acetylcholinesterase
BChE- Butyrylcholinesterase
BDNF- Brain derived neurotrophic factor.
CAT- Catalase
GSH-Px- Glutathione peroxidase
MDA- Malondialdehyde
SOD- superoxide dismutase
TNFα- Tumour necrosis factor α

INTRODUCTION

Glioblastoma Multiforme (GBM) arises from the glial cells of the Central Nervous System (CNS) and belongs to the astrocytoma subtype [1, 2]. Because the median survival period following diagnosis for glioblastoma multiforme is just 12 months-15 months, tis considered a serious disease [3]. The main causes of poor prognosis for glioblastoma multiforme are the late stage of diagnosis as well as the ineffectiveness of currently available treatments [4, 5]. Significant advances in the understanding of cancer biology over the past decades have led to extensive research within new classes of anticancer drugs, exploiting the molecular differences between cancer cells and healthy tissue cells [6].

Initially, only a patient biopsy's neuropathological study was used to diagnose CNS malignancies. Genotype and phenotypic characteristics have been added to the WHO classification in recent years, enabling a more accurate and powerful diagnosis of brain tumors [7].

Deregulation of a cell cycle G1/S checkpoint and the emergence of several genetic abnormalities in tumor cells are both factors in the progression of glioblastoma multiforme. Treatment options and prognosis are influenced by factors such tumor location, malignancy grade, genetic make-up, proliferative activity, patient age, and Karnofsky performance scale score [8].

To determine if ACh signaling has an impact on the biology of GBM, the role of AChE receptors (AChRs) were Choline acetyltransferase and choline transporters were jointly investigated, according to data from the Molecular Brain Tumor Data Repository (REMBRANDT), which indicated that GBM expresses all

proteins required for the generation and release of AchE [9].

GBM often develops in the subcortical white matter and is localized in the temporal, parietal, frontal, and occipital lobes [10]. These tumors are usually spread by "Cerebral Spinal Fluid (CSF)" and can be found at the cell level, scattered up to 2 cm-3 cm from their borders [11]. Thus, acetylcholinesterase is a naturally occurring component of the plasma membranes of neurons (as well as the endoplasmic reticulum and synapses), but its concentration varies greatly across various types of neurons and between different regions of the same type of neuron. The cholinesterase family of enzymes has a large role in a range of body functions. Acetylcholinesterase (AChE) is essential for central nervous system functions, while (BChE) is an enzyme that hydrolyzes many different choline esters [12].

Through various forms of nuclear DNA damage, studies have shown some evidence that Reactive Oxygen Species (ROS) contribute to the expansion of cancer by affecting antioxidant enzymes. These enzymes are involved in protecting against oxidative damage to biomolecules, and the ability to stop it, or reduce its severity, and so several antioxidant defenses have been developed, including the enzymes (SOD), (GSH-Px), and (CAT) [13, 14].

The DNA base modification (8-OH-dG), which results from deoxyguanosine oxidation, is thought to be the most accurate marker of oxidative DNA damage [15]. The overall balance of antioxidant defenses and pro-oxidant forces determines efficiency of antioxidant systems in protecting cells from oxidative damage [16].

In our study, we will examine the effect of glioblas- toma multiforme on cholinergic enzymes levels and examined the concentration of antioxidant enzymes and lipid peroxidation in serum of patients with glioblastoma multiforme tumor. Understanding the molecular details of these parameters enables us to identify potential drug targets as critical tasks for improving treatment [17].

MATERIALS AND METHODS

The study included taking blood samples during the routine examination from40 individuals divided into two groups, 20 of whom were diagnosed with GBM and 20 healthy individuals (Table 1 and 2), as a result of the examination performed in the Department of Neurosurgery at Van Yuzuncu Yil University Hospital. Laboratories facilities and equipment of Van Yuzuncu Yil University were employed for the research. The serum was separated after centrifuging the blood obtained during the regular examination at 5000 rpm for 5 minutes in a Nüve NF 800 centrifuge. The serum was maintained at -80 until the analysis was performed

Tab. 1. Data of patients with GBM

Patient No	Gender	Age	Other treatment	Taking chemotherapy
1	male	54	No	No
2	male	90	No	No
3	male	23	Radiotherapy	Yes
4	female	41	No	Yes
5	female	33	No	Yes
6	male	55	No	No
7	male	59	Radiotherapy	Yes
8	male	83	Radiotherapy	Yes
9	male	58	No	Yes
10	female	30	No	Yes
11	female	48	Radiotherapy	Yes
12	female	47	No	Yes
13	female	46	No	Yes
14	female	49	No	Yes
15	female	42	Radiotherapy	Yes
16	male	56	No	Yes
17	male	76	No	No
18	female	17	Radiotherapy	Yes
19	male	67	No	Yes
20	male	40	No	Yes

Tab. 2. Data of control group

Control group No	Gender	Age (years)
1	female	51
2	female	82
3	female	25
4	male	38
5	male	35
6	female	51
7	female	62
8	female	85
9	female	59
10	male	29
11	male	45
12	male	52
13	male	44
14	male	51
15	male	40
16	female	55
17	female	72
18	male	9
19	female	70
20	female	41

Biochemical analysis

AChE/ BChE enzyme activity:

AChE and BChE enzymes were determined spectrophotometrically 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'- dithiol-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 [6]. The intensity of this yellow color is directly proportional to the AChE/ BChE enzyme activity.

Measurement of BDNF:

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 is 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.

Measurement of apoptosis (Caspase-3) level:

Caspase-3 enzyme activity was determined in the control and experimental group samples using the "Caspase-3 Analysis Kit" 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.

Measurement of TNF-α level:

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.

Measurement of 8-OHdG Level:

Serum 8-OHdG was measured using an ELISA Kit. Half an hour before the start of the study, kit materials (Standard, Standard diluent, Microelisa Strip Plate, Str-HRP-Conjugate Reagent, 30X Wash solution, Biotin-(8-OHdG) Ab, Chromogen Solution A, Chromogen Solution B, Stop Solution) were brought to room temperature.

Measurement of MDA level:

200 µl of serum was transferred into a tube. 25 µl of BHT solution, 800 µl of phosphate buffer and 500 µl of 30% TCA were added. The tubes were mixed by vortex and kept in an ice bath for 2 hours after the caps are closed. Then, the tubes were brought to room temperature. After removing the caps of the tubes, they were centrifuged at 2000 rpm for 15 minutes. A total of 1 ml of the centrifuge's supernatant (filtrate) was extracted and put into additional tubes. 75 µl of EDTA and 25 µl of TBA were added to the filtrate, 1 ml of which is taken. After being vortex mixed, the tubes were placed in a 70°C hot water bath for 15 minutes. Then, it was brought to room temperature and the absorbances were read in the UV/Vis spectrophotometer at 532 nm.

Statistical analysis

The mean values and standard error (s.e.m) of the mean for the data were reported. Statistical Package for Social Sciences (SPSS) version 23.0 Inc. was used to evaluate statistical data differences at p<0, 05.

RESULTS

In this study, we examined changes in the levels of cholinergic system enzymes (AChE, BChE) and antioxidant enzymes (SOD, GSH-Px, CAT), BDNF, TNF-Alpha, Caspase-3, MDA, and 8-OHdG in the serum of patients with glioblastoma multiforme. We evaluated the serum levels of these parameters in GBM patients with the control group and then compared the results. Data were announced as mean values ± standard error of the mean (s.e.m). Statistically significant differences were contemplated significant at p<0.05. Statistical analysis of all data was achieved using SPSS (version 23.0 Inc).

SOD, GSH-Px and CAT activities in patients with GBM:

The SOD, GSH-Px, and CAT (u/mg) levels for the GBM patients compared to the control group were (34,909), (38,636) and (10,286) respectively (Figure 1). These values of SOD, GSH-Px, and CAT decrease in GBM patients' serum, and this change is statistically significant (*p<0.05).

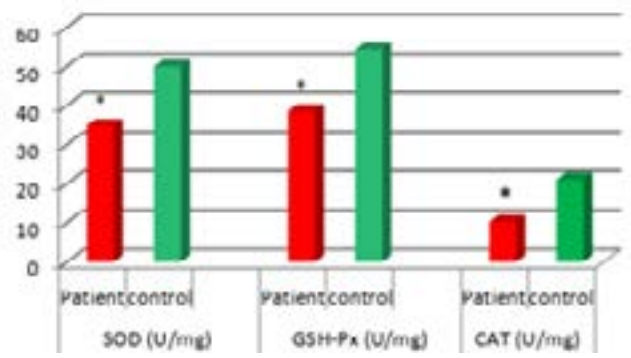

Fig. 1. (SOD, GSH-Px, and CAT) levels in the serum blood of GBM patients and the control group. Values are mean ± SEM *p<0.05.

AChE and BChE activities in patients with GBM:

The AChE and BChE (U/mg) levels for the GBM patients compared to the control group were (0,224) and (0,378) respectively (Figure 2). These values of AChE and BChE decrease in GBM patients' serum, and this change is statistically significant (*p<0.05).

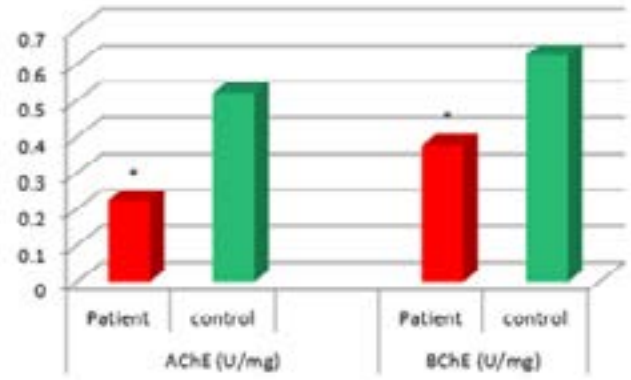

Fig. 2. AChE and BChE levels in the serum blood of GBM patients and the control group. Values are mean ± SEM *p<0.05

MDA and caspaz-3 activities in patients with GBM:

The MDA (nmol/mg) and caspase-3 (ng/ml) levels for the GBM patients compared to the control group were (10,162) and (5,035) respectively (Figure 3). These values of MDA and caspase-3 increase in GBM patients' serum, and this change is statistically significant (*p<0.05).

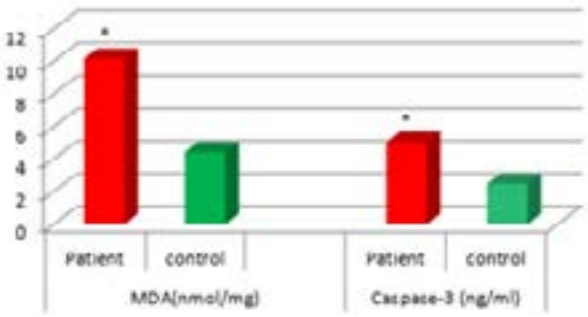

Fig. 3. MDA and caspase-3 levels in the serum blood of GBM patients and the control group. Values are mean ± SEM *p<0.05.

BDNF, TNF alfa and 8-OHdG activities in patients with GBM:

The BDNF, TNF-α and 8-OHdG (ng/ml) levels for the GBM patients compared to the control group were (276,825), (281,868) and (148,037) respectively (Figure 4). These values of BDNF, TNF-α and 8-OHdG increase in GBM patients' serum, and this change is statistically significant (*p<0.05).

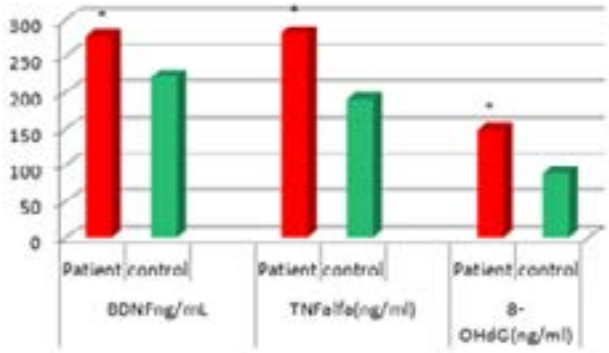

Fig. 4. BDNF, TNF-α and 8-OHdG levels in the serum blood of GBM patients

DISCUSSION

To combat oxidative stress, our bodies contain an internal defense mechanism made up of natural antioxidant enzymes. These include the vital enzymes SOD, GSH-Px, and CAT, which work in concert in human cells to combat harmful reactive oxygen species and have a wide range of pathophysiological processes and possible therapeutic benefits. ROS produced during metabolism can participate in mechanisms that harm particular types of human cell activities. Peroxidative modifications to membranes and other biological components, including oxidative DNA damage, are examples of direct impacts. Before superoxides and peroxides combine with metal catalysis to create new reactive species, intracellular SOD, GPX, and CAT eliminate them [18].

ROS have a significant role in the development of cancer and neurological diseases. Because of the damage, they do to DNA, oxygen species (ROS) have been accepted as a major cause of cancer [19]. Consequently, tissues need to be protected from this oxidative damage by expressing stress response genes, genes for antioxidant enzymes, and by turning on other relevant transcriptional regulatory proteins.

GSH-Px, SOD, and CAT levels were all lowered in patients with GBM, respectively; therefore it stands to reason that tumors with high levels of SOD and GSH-Px would have less of an inhibitory influence on CAT activity. These enzymes guard against the harm that free radicals may do to cells. The superoxide radical (O2-), which catalyzes the dissociation process, is eliminated by SOD. CAT and GSH-Px, which shields the cell from the generation of more harmful hydroxyl radicals in the Fenton or Haber-Weiss reaction, carry out the dissociation of H2O2 [20].

Recent research clearly suggests that Neural Stem Cells (NSC) found in the adult brain is the origin of glioblastoma multiforme. Neurotransmitters and their receptors have been revealed to have a significant role in the understanding of the mechanisms that govern and maintain NSC in the adult brain [21, 22].

Several bodily processes include the cholinesterase family of enzymes. While (AChE) is a crucial cholinesterase enzyme for CNS functioning, (BChE) is a general cholinesterase enzyme that hydrolyzes a variety of choline-based esters [23]. According to several research, AChE and BChE expression levels rise in various cell types during apoptosis. Apoptosis or stimulated states greatly increase AChE and BChE expression levels [24]. Our findings on the AChE activity in tumor cells indicate that this enzyme has poor activity in glioblastoma multiforme. It appears that BChE activity in the blood plasma of individuals with glioblastoma multiforme is somewhat higher relative to AChE, even though BChE occurs alongside AChE and that BChE reactivity may differ from that of AChE. Cholinergic enzymes are a novel regulator in cell proliferation and cell death; this allows its use as a potential marker for cancer diagnosis and prediction.

Low molecular weight MDA is an aldehyde that waa created when polyunsaturated fatty acids are attacked by free radicals. Increased (ROS) that can cause oxidative stress, molecular damage, including lipid peroxidation, and overexpression of MDA in GBM patients. Cancer growth and treatment resistance frequently exhibit elevated ROS levels and downregulation of redox signaling. MDA may interact with proteins and DNA to result in gene alterations that give rise to tumor cells, and elevated levels of MDA are an indication of the development of cancer cells [25]. Our results showed high Lipid Peroxidation expression (MDA), providing molecular evidence that MDA can cause cell death in GBM.

Molecular events associated with apoptosis, including responsive caspase-3, have been demonstrated in U87 GBM xenografts [26]. Caspase-3 was expressed in more than 50% of GBM tumor cells, according to a prior immunohistochemistry research [27]. A fresh viewpoint has been provided by research on the role of caspases, particularly caspase-3, in cancer cells. It has been demonstrated that GBM cells are the main source of active caspases, which increase the motility of these cells in the absence of cellular stress. Human GBM samples, newly separated GBM cells, and long-term maintained glioma cell lines all had somewhat high amounts of activated caspase-3 [28].

Since caspase, activity is often required for apoptosis to take place; a complex regulatory mechanism keeps normally dividing cells from going through this process [29]. Recent research shows that apoptosis is the primary cause of cell death in malignant gliomas. The development, progression, and poor response of tumors to

therapeutic approaches in brain cancer are all impacted by dysregulation of apoptotic pathways [30, 31]. in our study we observed lower protein expression of caspase-3 compared to the expression of MDA, suggesting of oxidative stress and the antioxidant status in GBM patients. Plasma MDA and caspase-3 appear to reflect the oxidative stress status these may serve as indicators for the progression of oxidative stress and illness.

Our study also showed in the brain, levels of TNF-α, BDNF and, 8-OHdG are elevated under GBM, and thus its effect on glioma cell growth and apoptosis. These parameters are member of the neurotrophic superfamily; that play important role in the pathophysiology of the nervous system. Levels of TNF-α, BDNF and, 8-OHdG, which reflects Oxidative DNA damage, were significantly higher in patients with GBM than in normal brain.

One of the most researched and thoroughly defined neurotrophic factors in the Central Nervous System (CNS) is the neurotrophic BDNF. It binds to and activates the Tropomyosin Receptor Kinase B (TrkB), a member of the vast family of Trk receptors, which regulates a variety of cellular processes involved in the development and maintenance of proper brain function. Glial cells, including microglia and astrocytes purified from the cortex and hippocampus, in the brain express BDNF [32]. BDNF-TrkB signaling encourages the differentiation of cortical progenitor cells throughout embryonic development, which then encourages the differentiation of cortical progenitor cells into neurons. Numerous investigations have shown that BDNF/TrkB signaling plays a role in adult neurogenesis [33]. Recent research has demonstrated that BDNF generated by differentiated glioblastoma cells (GBM) works on GBM stem cells, stimulating their development through paracrine signaling [34, 35].

CONCLUSION

This study is a comprehensive characterization study to investigate the level of change in (AChE, BChE, BDNF, TNF-Alpha, Caspase-3, 8-OHdG) in patient serum of glioblastoma multiforme. It has been noted that people with Glioblastoma Multiforme (GBM) display almost lower levels of acetylcholinesterase, Butyrylcholinesterase, .SOD, GSH-Px, and CAT) and higher levels of (MDA, Caspaz-3, BDNF, TNF alfa, and 8-OHdG), Thus, results will enable the use of the parameters specified in Glioblastoma Multiform Disease as markers in determining the aggressiveness of the tumor, As well as a better knowledge of anti-apoptotic and pro- apoptotic proteins and thus the ability to develop new therapies based on a molecular basis.

STATEMENTS AND DECLARATIONS

Ethics approval: Van Yüzüncü Yil University Permission was obtained by the Non-Interventional Clinical Research Ethics Committee with the letter dated 04/12/2020 and decision number 2020/09-20.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHORS CONTRIBUTIONS

All authors participated in the writing of the manuscript, read and approved its final version.

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