Original Investigation

Tissue Thiol Concentration in High-Grade Gliomas: Is There any Association Between IDH1 Mutation Presence and Tumoral Cellular Antioxidant Defense?

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ABSTRACT

AIM: To assess and compare the antioxidant capacities of high-grade gliomas (HGG) according to their grades and the presence of isocitrate dehydrogenase 1 (IDH1) mutation using tissue thiol level measurement.

MATERIAL and METHODS: Tissue thiol concentrations were measured in 41 HGG samples and 21 healthy brain tissues obtained from autopsy procedures, which were performed within the first 4 hours of death. All samples were stored at –80°C, and a thiol quantification kit was used in evaluating tissue thiol levels. The Number Cruncher Statistical System was used for statistical analyses to detect the differences between the control group and the HGG group, which was also divided into subgroups according to their grade and IDH1 mutation presence.

RESULTS: The tissue thiol levels of HGGs were found to be higher than the control group (p=0.001). Although the median thiol levels of Grade 4 gliomas were higher than those of Grade 3, no statistically significant difference was noted (p=0.076). When all tumors were compared according to the IDH1 mutation presence, IDH1-negative (IDH1-) HGGs had higher thiol contents than IDH1 mutant (IDH1+) HGGs (p=0.001). The thiol levels of Grade 4 IDH1- gliomas were statistically significantly higher than of Grade 3 gliomas (p=0.023), but no statistically significant difference between the thiol levels of Grade 3 and Grade 4 IDH1+ tumors was noted (p=0.459).

CONCLUSION: We have demonstrated the higher thiol concentrations of HGGs, particularly IDH1- ones. The sulfhydryl contents of gliomas as an indicator of tumoral antioxidant capacity may be responsible for the treatment resistance of IDH1- gliomas, the mechanism of which is not clear. Thiols can be a novel target for treatment, considering the unsatisfactory results of current modalities for HGGs.

KEYWORDS: High-grade glioma, Glioblastoma, IDH1, Thiol, Antioxidant

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◼ INTRODUCTION

High-grade gliomas (HGG) comprise the World Health Organization (WHO) Grade 3 and Grade 4 gliomas, which are seen predominantly in 50–60-year-old men (32). Grade 4 glioma, which is also called "glioblastoma (GBM)," is the most common primary malignant brain tumor in adults and, despite the new treatment options, has poor prognosis, with a median survival ranging between 12 and 15 months (1). GBM can be divided into primary and secondary, which arises de novo and transforms from a lower grade glioma, respectively (31).

Isocitrate dehidrogenase 1 (IDH1) enzyme, which belongs to the Krebs cycle, catalyzes the reversible oxidation of isocitrate to procure α-ketoglutarate and nicotine adenine disphosphonucleotide (NADPH) (10). NADPH inhibits intracellular oxidative damage (22). IDH1 mutations were first described for GBMs in 2008 (25), which were found to be related with better prognosis and longer survival in the whole spectrum of gliomas (15). While the mutation of IDH1 occurs rarely in primary GBM, it has been reported in up to 85% in secondary GBM, which has better prognosis than primary GBM (40).

Thiols, also known as "mercaptans," are compounds that contain a functional sulfhydryl (-SH) group, which comprises a hydrogen atom and a sulfur atom (35). Since the sulfhydryl group acts as a major target for oxidant compounds, thiols are responsible in neutralizing oxidative stress by forming disulfide (-SS) bonds. Although thiols take a part in signal transduction, metabolism, proliferation, and cell death processes (2), they are the main determinant factors in assessing the total antioxidant capacity (TAC) of cellular homogenates with their antioxidant functions (3). Measurement of tumor's antioxidant capacity and modifications of sulfhydryl group have become a current issue for cancer treatment (5,12).

Chemotherapy and radiotherapy, which are the treatment options for HGGs, can be used to induce the overproduction of reactive oxygen species (ROS), which cause oxidative stress and apoptosis of tumor cells (41). Thus, the cancer cells can produce more antioxidants to gain resistance against chemotheraphy or radiotherapy (29). Limiting the cellular antioxidant defense system of tumors reduces their neutralizing ability of oxidative damage, which makes them vulnerable to oxidative stress, resulting in tumor cell death. In this respect, investigating the compounds, which cause a higher antioxidant capacity of tumors, can be useful for increasing the effectiveness of treatment and finding better treatment options for incurable tumors especially GBMs.

In this study, we investigated the concentration of thiols, which are the main antioxidant compounds of intracellular milieu, and its distribution in different HGG grades. Also, we researched whether any association exists between tumor's cellular thiol content and IDH1 mutation, which is a prognostic factor for gliomas.

◼ MATERIAL and METHODS

This prospective study was performed in collaboration with Haseki Training and Research Hospital's Neurosurgery Clinic and Istanbul Training and Research Hospital's Biochemistry Department. Patients with a histopathologic diagnosis of HGG, consisting of the WHO Grade 3 or Grade 4, were included in the study. They, along with their relatives, were informed in detail, and those that signed the informed consent were included. Ethics committee approval was obtained from the local ethics committee, and our study was planned in accordance with the Declaration of Helsinki.

Patient Population

A total of 41 patients who had their first surgery due to HGG between the years 2017 and 2019 were included. Patients who had recurrent surgery were excluded from the study to eliminate oxidant effects of radio- and chemotherapy on tissues. Patients who had any other neoplastic diseases, signs of infection, and recent major surgery history and did not want to contribute to the study were excluded from the study. All participants were evaluated in our clinical preoperative case meetings. Contrasted brain magnetic resonance imaging (MRI), routine blood workup, and other needed specialty consultations were done. All patients and their relatives were informed about the benefits and risks of surgery preoperatively and signed the informed consent form. All operated patients were followed up at regular intervals after surgery. Pathology reports were assessed to confirm HGG diagnosis and note the IDH1 mutation presence. IDH1 mutated and nonmutated tumors were referred as IDH1 mutant (IDH1+) and IDH1-negative (IDH1-), respectively.

Control Samples

The control samples were obtained from people autopsy series in the Department of Forensic Medicine. Poisoning and the cases with any neoplastic, inflammatory, or other chronic diseases, which were detected during autopsy, were excluded from the study. Also, the specimens with a sign of any kind of brain lesions were excluded from the study. The control group consisted of 21 people, 11 of whom died in traffic accidents, 6 by falling from height, and 4 by shot gun assault.

Specimen Handling

Glioma samples were procured from patients during surgery. Attention was paid for taking samples from the most viable parts of tumors, avoiding necrotic and cystic parts, which were confirmed with preoperative radiological investigations. The samples were divided into two same-sized portions. The first portions of these samples were fixed in 10% formalin for pathological investigations, and the others were frozen at -80^0C for biochemical analyses. Healthy glial tissues were extracted from the control group during autopsies, which were performed within the first 4 hours of death. All of the obtained samples taken from control group were stored at $-80°$C for upcoming biochemical analyses.

Sample Preparation

Tissue samples were thawed at room temperature, which have been preserved at $-80°$C previously. They had been weighed on a precision scale and phosphate tamponade (PBS, 0.1 M, pH=7.4) added with 1:9 ratio. Samples were homogenized

using MICCRA, Germany homogenisator. Samples were kept in ice to avoid heat that cause protein loss. After centrifugation at 5,000 rpm for 5 minutes, supernatants were divided into equal portions for two different runs.

Biochemical Analysis

Protein and thiol measurements had been performed in these supernatants. Protein levels were evaluated as pair by a commercially available biochemistry auto-analizator (Beckman Coulter, AU5800, USA) with calorimetric method. Thiol levels were measured using the thiol quantification kit (Innova Biosciences, Catalog No: 418-0002). All measurements have been applied upon the manufacturer's request. All samples have been worked as a pair. Thiol concentration was measured per mg protein to eliminate errors caused by protein loss. The detecting range of thiol kit was 5–250 μM.

Immunohistochemical Analyses

4-micrometer-thick (4 micron) sections of formalin-fixed paraffin embedded tissues were placed on 3-aminopropy-letxylene-covered slides. Subsequently, they were stained with rabbit polyclonal Dianova IDH1 R132H antibody (1/20 titer; H09 clone, mouse monoclonal antibody) following the manufacturer's protocol. Briefly, staining was performed on the Ventana Bench Mark Ultra (Ventana Medical Systems Inc.).

The staining protocol included cell conditioning 1 for 60 minutes, pre-peroxydase inhibition, and primary antibody incubation for 40 minutes at 37°C, applying the amplification kit (Ventana Medical Systems Inc.) for 4 minutes at 37°C to increase the signal intensity. UltraView Universal DAB Detection Kit (Ventana Medical Systems Inc.) was used to detect Isocytrate dehydrogenase 1 (IDH1) protein expression. Tissues were counterstained with hematoxylin for 16 minutes and bluing reagent for 4 minutes.

Statistical Analyses

The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) program was used for statistical analyses. Definitive statistical methods (mean, standard deviation, median, first quartile, third quartile, frequency, minimum, maximum) were used to assess the study data. Comparing the quantitative variables that did not show normal distribution between the two groups was achieved by the Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was used to determine the cutoff. $P < 0.05$ was considered statistically significant.

■ RESULTS

This study consists of mainly two groups: HGGs and controls. The HGG group was divided into subgroups according to their grades and IDH1 mutation existence to investigate the relation between thiol levels, grades, and IDH1 mutations.

The HGG group included 14 (34.1%) women and 27 (65.9%) men, with a mean age of 46.73 ± 10.6 years (range, 27 to 67 years). The control group had 8 women and 13 men, with a mean age of 44.42 ± 12.02 years (range, 21 to 73 years).

The HGG group was separated into two subgroups according to the WHO grades of tumors. Grade 3 HGG group consisted of 18 patients, with a mean age of 40.27 ± 17.7 years (range, 27 to 54 years), 6 (33.3%) of whom were women and 12 (66.7%) men. The most common symptom was headache (47.7%), followed by seizure (33.3%) and weakness (19.0%). Most of the Grade 3 gliomas were located at the right hemisphere (77.8%) and frontal lobe (55.5%).

In the Grade 4 HGG group, there were 23 patients, with a mean age of 51.78 ± 7.1 years (range, 41 to 67 years), 8 (34.8%) of whom were women and 15 (65.2%) men. The most common finding of Grade 4 glioma was headache (48.3%), followed by weakness (17.3%), seizure (17.3%), vertigo (10.3%), visual problems (3.4%), and loss of consciousness (3.4%). While most of the Grade 4 gliomas were located at right hemisphere (78.2%), the temporal lobe had a slightly higher incidence from the other lobes for tumor presence. Table I summarizes the detailed clinical characteristics of the HGG group.

IDH1 mutation was found to be positive in 8 (44.5%) of 18 Grade 3 glioma and 11 (47.8) of 23 Grade 4 glioma patients. A total of 19 patients (46.3%) had IDH1 mutation, whereas 22 (53.7%) did not.

Median tissue thiol levels of HGG and control groups were 0.18 (0.09, 0.27) and 0.07 (0.06, 0.08), respectively. Higher median thiol level of patient group than control group was found to be statistically significant (p=0.001) (Table II) (Figure 1).

Thiol levels of HGG group were investigated separately according to the grades. While the median level of Grade 3 tumors was 0.16 (0.09, 0.23), Grade 4 tumors was 0.25 (0.11, 0.31). Although the individual tissue levels of both Grade 3 and Grade 4 tumor groups were higher than the control group with statistical significance (p=0.001), a higher median thiol level of the Grade 4 group than the Grade 3 group was not statistically significant (p=0.076) (Table III) (Figure 2).

Tissue thiol levels were analyzed regardless of the Grade of the tumor according to the IDH1 mutation presence. The median tissue level of IDH1+ tumors was 0.09 (0.07–0.13), while that of the IDH1- tumors was 0.25 (0.18–0.31), with a statistically significant difference between the two groups (p=0.001) (Table IV) (Figure 3).

Both Grade 3 and Grade 4 HGG groups were divided into two subgroups to investigate the association between IDH1 mutation and tissue thiol levels. In the Grade 3 HGG group, median tissue thiol levels of IDH1+ and IDH1- subgroups were 0.09 (0.07, 0.11) and 0.22 (0.17, 0.26), respectively (p=0.001). Also, the median tissue levels were 0.11 (0.06, 0.25) for IDH1+ and 0.30 (0.24, 037) for IDH1- subgroups in the Grade 4 HGG group. The higher levels of IDH1- subgroups than the IDH1+ subgroups were found to be statistically significant in both Grade 3 and Grade 4 HGG groups (p=0.001 for Grade 3, p=0.002 for Grade 4).

The tissue thiol concentrations of both IDH1- and IDH1+ tumors were compared according to the grade of tumor. The difference between median levels of Grade 3 and Grade 4 IDH1+ tumors was not statistically significant (p=0.459). In

Table I: Clinical Characteristics of the High-Grade Glioma Patients

High-grade gliomas (n=41)	Grade 3 (n=18)	Grade 4 (n=23)
Mean Age (years)	40.27 ± 17.7 (range: 27 - 54)	51.78 ± 7.1 (range: 41 - 67)
	n (%)	**n (%)**
Sex (F, M)	6 F (33.3) 12 M (66.7)	8 F (34.8%) 15 M (65.2%)
Symptoms	Headache (47.7) Seizure (33.3) Weakness (19.0)	Headache (48.3) Weakness (17.3) Seizure (17.3) Vertigo (10.3) Visual problems (3.4) LOC (3.4)
Lesion side	Right: 14 patients (77.8) Left: 4 patients (22.2)	Right: 18 patients (78.2) Left: 5patients (21.8)
Localization	Frontal; 10 patients (55.5) Frontoparietal; 5 patients (27.8) Parietal; 2 patients (11.1) Temporal; 1 patients (5.6)	Temporal; 8 patients (13.7) Parietal; 6 patients (9.1) Temporoparietal; 6 patient (4.5) Frontal; 3 patients (9.1)
Multiple surgeries	6 patients (33.3)	5 patients (21.8)
Resection	4 Subtotal (27.8) 14 Total	7 Subtotal (30.4) 16 Total (69.6)
IDH1 Mutations	**10 IDH1- (55.5)** **8 IDH1+ (44.5)**	**12 IDH1- (52.2)** **11 IDH1+ (47.8)**

IDH1+: Isocitrate dehydrogenase 1 mutant, IDH1-: Isocitrate dehydrogenase 1 negative, LOC: Loss of consciousness.

Table II: Tissue Thiol Concentrations of Control and Patient Groups

	Thiol	
	Median (Q1, Q3)	**p**
Control	0.07 (0.06, 0.08)	**0.001**
Patients	0.18 (0.09, 0.27)	

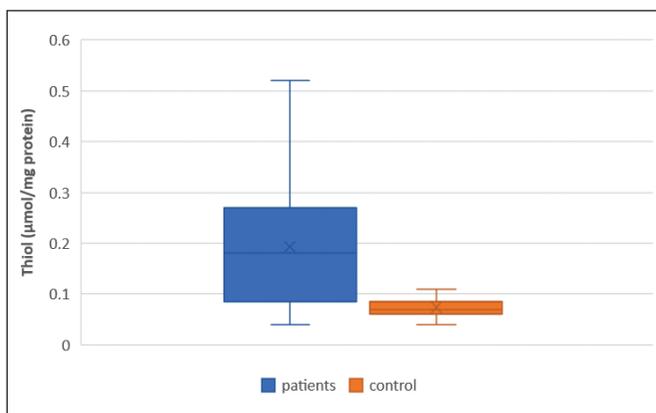*Mann-Whitney U test. Q1: First quartile, Q3: Third quartile*

Figure 1: Distribution of tissue thiol concentrations in patient and control groups.

Table III: Tissue Thiol Concentrations of Grade 3, Grade 4 Gliomas and Control Groups

	Thiol
	Median (Q1, Q3)
Control	0.07 (0.06, 0.08)
Grade 3	0.16 (0.09, 0.23)
Grade 4	0.25 (0.11, 0.31)
p (Grade 3 vs Control)	**0.001**
p (Grade 4 vs Control) **p (Grade 3 vs Grade 4)**	**0.001** 0.076

Mann-Whitney U test. Q1: First quartile, Q3: Third quartile.

contrast, IDH1- Grade 4 tumors had higher thiol levels, with statistically significance, than IDH1- Grade 3 tumors (*p*=0.023) (Table V) (Figure 4).

ROC analysis was performed to determine the cutoff of thiol value in differentiating glioma and control groups. The area under the curve obtained from the evaluation was found to be 0.821 [AuROC (95% CI): 0.821 (0.718, 0.923), *p*=0.001], while the thiol value was ≥ 0.12, sensitivity 65.85%, specificity 100%, PPV 100, and NPV 60 (Figure 5).

ROC analysis was performed to determine the cutoff of thiol value to differentiate IDH1+ and IDH1- groups. The area under the curve obtained from the evaluation was found to be 0.893 [AuROC (95% CI): 0.893 (0.788, 0.998), p=0.001]. For the case where the thiol value was ≥0.14, the sensitivity was found to be 100%, specificity 78.9, PPV 84.6, and NPV 100 (Figure 6).

cancer in healthy organism, they also can facilitate the survival of tumoral tissue, forming chemo- or radioresistancy. At this point, thiols seemed to be a double-edged sword. Understanding the mechanism of their different behavior at different tumor types via possible different pathways may be beneficial for finding out new treatment strategies.
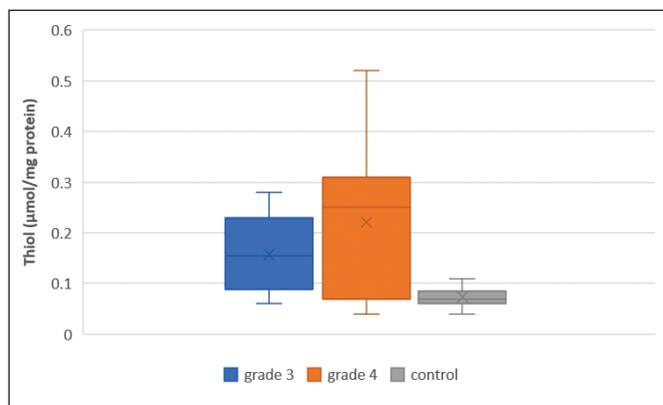
■ **DISCUSSION**

Thiols are one of the most important antioxidant molecules for living organisms. While their antioxidant effects prevent

Figure 2: Distribution of tissue thiol concentrations in Grade 3 and Grade 4 glioma and control groups.

Table IV: Comparison of Tissue Thiol Concentrations According to IDH1 Mutation Presence

	Thiol	
	Median (Q1, Q3)	**p**
IDH1+	0.09 (0.07, 0.13)	**0.001**
IDH1-	0.25 (0.18, 0.31)	

IDH1+: *Isocitrate dehydrogenase 1 mutant,* *IDH1-:* *Isocitrate dehydrogenase 1 negative. Mann-Whitney U test.* **Q1:** *First quartile,* **Q3:** *Third quartile.*
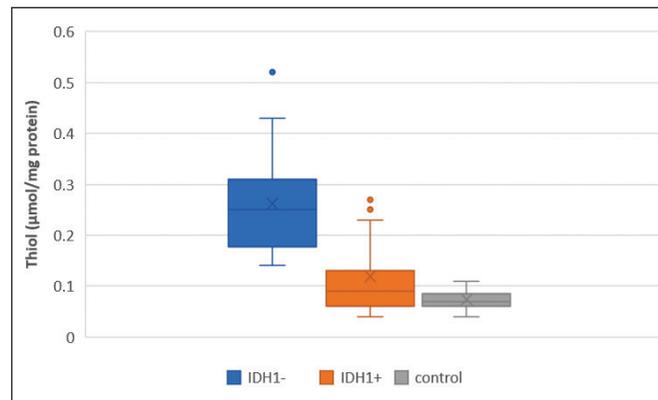
Figure 3: Distribution of tissue thiol concentrations in IDH1 mutant (IDH1+), IDH1-negative (IDH1-), and control groups.
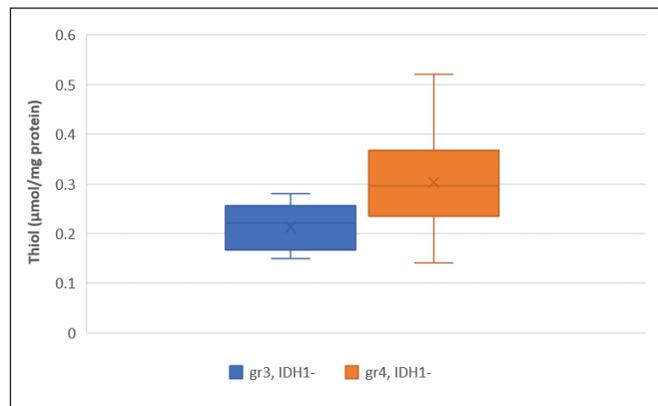
Figure 4: Distribution of tissue thiol concentrations in IDH1-negative (IDH1-) tumors according to grades.

Table V: Tissue Thiol Concentration according to Grades and IDH1 Mutation Presence

		Thiol	
		Median (Q1, Q3)	**p**
Grade 3	IDH1-	0.22 (0.17, 0.26)	**0.001**
	IDH1+	0.09 (0.07, 0.11)	
Grade 4	IDH1-	0.30 (0.24, 0.37)	**0.002**
	IDH1+	0.11 (0.06, 0.25)	
		p [Grade 3 IDH1- vs Grade 4 IDH1-]	**0.023**
		p [Grade 3 IDH1+ vs Grade 4 IDH1+]	0.459

IDH1+: *Isocitrate dehydrogenase 1 mutant,* *IDH1-:* *Isocitrate dehydrogenase 1 negative. Mann-Whitney U test.* **Q1:** *First quartile,* **Q3:** *Third quartile.*
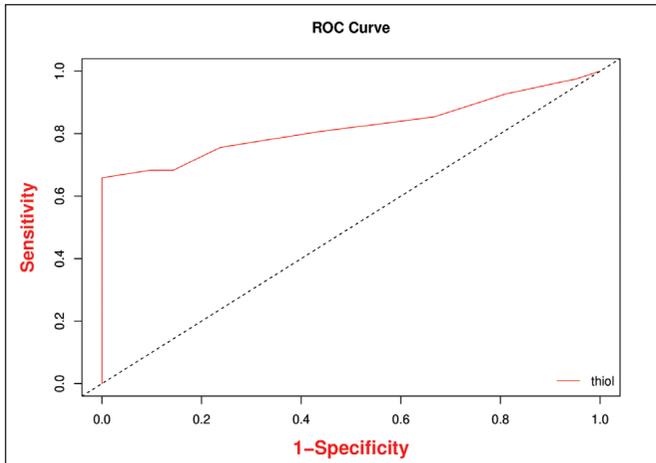
Figure 5: The ROC curve analysis of tissue thiol levels in glioma and control groups.
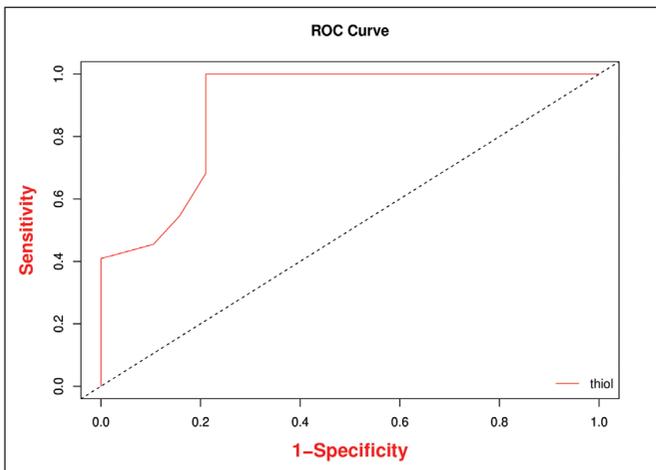

Figure 6: The ROC curve analysis of tissue thiol levels in IDH1 mutant (IDH1+) and IDH1 negative (IDH1-).

One of the immunoresponsive processes is related to an enzyme called as gamma-interferon-inducible lysosomal thiol reductase (GILT), which includes a sulfhydryl group and has a disulfide bond reduction function in the antigen presentation process (16,26). While a lower expression of GILT was found to be connected to a worse prognosis of diffuse large B-cell lymphoma patients (34), it has a higher expression for melanoma patients as previously reported (30). Eventually, Chen et al. researched this subject in cell cultured glioma tissues (6). Additionally, they found the higher expression of GILT in glioma cell lines, silencing GILT, which resulted in the suppression of proliferation, colony formation, migration, and tumor growth and the induction of cell cycle arrest and apoptosis. Although we are congruent with them in our results, the other molecules containing sulfhydryl groups may be have a part in this neoplastic process, which is not certainly clarified.

Nuclear factor kappa B (NF-κB) was associated with cancer previously (4). Linnewiel-Hermoni et al. investigated the effect of carotenoid derivates on NF-κB and its mechanism in both cancer and bone cells (23). They reported that the carotenoid derivates inhibited NF-κB pathway by using specific thiol groups of p65 and IκB kinase (IKK), which are the two key proteins of this pathway. As a consequence, they suggested the carotenoid derivates contribute to the prevention of cancer. When the poor prognosis of HGGs and their higher tissue thiol concentration are considered, thiol-targeted therapy modalities can ensure a better prognosis. For this purpose, finding the molecules that are exceeding the blood brain barrier or are usable in the surgical procedures, which can be achieved by understanding the mechanism via further studies in HGGs, might be helpful.

Deorukhkar et al. investigated the effects of "zerumbone" which is a cyclic sesquiterpene derived from ginger plant (20), on colorectal cancer cell lines (8). Their in vitro study demonstrated that the zerumbone contributes to radiation-induced cell cycle arrest in G2/M phase and apoptosis. One of their gripping findings was maintaining of radiosensitization caused by the zerumbone achieved with depleted glutathione (GSH) levels but neither boosted the radiation-induced ROS generation nor generated ROS by itself. Our study discussed that the HGGs have higher thiol levels than healthy brain tissues. Molecules, which target the thiol groups of these tumors, can be one of the treatment options to increase survival and health quality.

Withaferin A is a steroidal lactone maintained from a medicinal plant called *Withania somnifera* and has anticancer impacts on various cancer types, such as myeloid leukemia (24), or renal cancer (7). Although withaferin A-induced apoptosis have been previously reported for head and neck carcinoma cells (36), Park et al. investigated the underlying mechanisms in vitro (33). They reported that the withaferin A-mediated apoptosis is independent of ROS production and cyclooxygenase-2 (COX-2) expression, which are related to different cancer types, such as melanoma (27), and renal cancers (14), respectively. Most importantly, they indicated that the thiol oxidation is an important mechanism for withaferin A-induced apoptosis and withaferin A may target cysteine residues of proteins, such as kinases and chaperons. In this current issue, the sulfhydryl groups, which have elevated levels in the HGGs from the normal brain tissues, also can be a target for novel molecules, which may be used in the HGG treatment. Further studies are also needed.

A high level of consumption of cruciferous vegetables, such as broccoli and cauliflower, reduces the risk of various cancer types previously reported (17). Anticancer effects of the cruciferous vegetables have been found to be related to their high glucosinolate content (9). Kim et al. investigated the effect of sulphoraphane, which is a glucosinolate precursor, on the ovarian cancer cells, indicating the anticancer effect of sulphoraphane decayed by thiol-reducing agents but not by the ROS-scavenging antioxidants (19). However, they did not explain the underlying mechanism, and they suggested further studies are needed for understanding these pathways. Similarly, in our study, higher levels of reduced thiol concentrations of glial tumors correlate with poor prognosis. At this point, higher concentrations of thiol levels especially in higher

grade and IDH1- subgroups may be one of the reasons of poor prognosis. Modalities, which target tumor's thiol content, may be a treatment option.

Previous studies have suggested the IDH1 mutations were found to be related with better prognosis and prolonged survival in glioma patients with different grades (21,37). IDH1 catalyzes the oxidative carboxylation of isocitrate to α-ketoglutarate, which is a reaction that produces NADPH (28, 42). Production of GSH from the oxidized form of glutathione (GSSG) is dependent to NADPH (18). Also, reduced levels of intracellular GSH, which is a thiol compound and the most abundant intracellular antioxidant, lead to increased ROS levels in cells, causing apoptosis resistance (11,13). From this point, Shi et al. investigated the inhibitor effects on tumor growth of elevated ROS levels and the depletion of GSH caused by IDH1 mutation (39). They concluded the inhibitor cell growth effect of IDH1 mutation may be caused by GSH depletion and ROS generation. Although they investigated GSH, which is a thiol compound, they collected data from the cell culture, while our study was human tissue and tumor based. At this point, both of these studies require further studies to understand certain mechanism of IDH1 mutation-related prognosis, especially from the tumoral antioxidant aspect.

Also, Shi et al. designed an additional cell culture-based study, wherein they investigated the relation between IDH1 mutation and chemosensitivity in gliomas (38). They pointed out the higher chemosensitivity of IDH1 mutant cell lines were associated with NADPH, GSH depletion, and elevated ROS generation. Our findings are parallel to theirs because of the higher thiol concentrations of IDH1- tumors, which indicate higher tumoral antioxidant capacity, chemoresistency, and worse prognosis. As a result, alternative treatment modalities, with mechanisms such as binding active thiol groups, may be an option for the treatment of these poor prognosed tumors.

Our findings indicate that HGGs, particularly IDH1- gliomas, have higher thiol concentrations than normal brain tissues. In this respect, decaying their antioxidant defense may be an option for improving the management of HGGs, which cannot be cured with current treatment options. Further studies are necessary to discover detailed mechanisms of antioxidant systems and how to modify them.

This study had some limitations. First, not all of the patients who had surgery in our clinic wanted to participate in the study, resulting in the restriction of our patient population. Also, all of the patients who could be involved in this study had been diagnosed histologically with Grade 3 or Grade 4 glioma, and consequently, we could not investigate the thiol content of low-grade gliomas. Further studies, which comprise a larger patient group, can be planned for more certain data.

◼ CONCLUSION

Thiols are the main antioxidant molecules of the intracellular area. However, their anti-oxidizing abilities have crucial roles for maintaining survival of healthy cells; they can contribute to resistance against treatment and cause worse prognosis in HGGs. Exploring the underlying mechanisms can be helpful in discovering new target selective treatment strategies based on the thiol group. In this respect, our study can provide beneficial knowledge for further studies.

◼ ACKNOWLEDGMENT

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