Turk Neurosurg 33(5):847-854, 2023

Original Investigation

DOI: 10.5137/1019-5149.JTN.42622-22.2

Received: 19.10.2022
Accepted: 28.12.2022

Published Online: 31.05.2023

Could Fluorescein Staining in Low-Grade Glial Tumors Guide for Peroperative Differentiation of Pathological Types?

Mahmut OZDEN[1], Orkhan MAMMADKHANLI[2], Murat ZAIMOGLU[3], Eyup BAYATLI[3], Melih BOZKURT[1,4]

[1]Private Memorial Hospital, Department of Neurosurgery, Istanbul, Turkey
[2]Trakya University, School of Medicine, Department of Neurosurgery, Edirne, Turkey
[3]Ankara University, School of Medicine, Department of Neurosurgery, Ankara, Turkey
[4]Arel University, School of Medicine, Department of Neurosurgery, Istanbul, Turkey

Corresponding author: Melih BOZKURT ✉ melihbozkurt@hotmail.com

ABSTRACT

AIM: To define whether characteristics of fluorescein staining help to distinguish low grade gliomas intraoperatively.

MATERIAL and METHODS: We studied 46 patients with supratentorial newly diagnosed noncontrast-enhancing LGGs removed by fluorescence guidance under the YELLOW 560 nm filter. Patients who were treated between July 2019 and 2022 were retrospectively analyzed. Clinical data were collected from patient records. Patients' intraoperative video recordings, pathological examination, and preoperative magnetic resonance imaging (MRI) were analyzed and compared for each patient after the operation. Histopathologically, patients were divided into WHO Grade 2 oligodendrogliomas, diffuse astrocytomas (IDH mutant, 1p19q negative tumors), and pre-glioblastomas (IDH wild type, 1p19q negative tumors). Resection margins were checked using controls contrast-enhanced cranial MRI at the postoperative 24 and72 hours.

RESULTS: Our observations indicate that fluorescein primarily stains diffuse astrocytomas (IDH mutant, 1p19q negative tumors) and pre-glioblastomas (IDH wild type, 1p19q negative tumors) rather than WHO Grade 2 oligodendrogliomas.

CONCLUSION: Fluorescein staining might be an option to determine tumor borders in WHO Grade 2 glial tumors, particularly for those with a higher malignancy potential.

KEYWORDS: Glioma, Fluorescein, Isocitrate dehydrogenase

ABBREVIATIONS: 5-ALA: 5-Amino levulinic acid, **BBB:** Blood brain barrier, **DIA:** Diffuse infiltrative astrocytoma, **EGFR:** Epidermal growth factor receptor, **FLAIR:** (T2) fluid attenuated inversion recovery, **GBM:** Glioblastoma multiforme, **GTR:** Gross total resection, **IDH:** Isocitrate dehydrogenase, **hTERT:** Human telomerase reverse transcriptase, **LGG:** Low grade glioma, **NE:** Non-enhancing, **WHO:** World Health Organization

INTRODUCTION

According to the recent WHO classification of brain tumors, molecular parameters overrule based on tumor pathomorphology (23). Currently, genotype takes priority in patients where the phenotype and genotype are not concordant (4). Oligodendrogliomas are glial tumors with 1p19q codeletion and IDH mutation (4). Grade 2 gliomas without 1p19q deletion but with IDH mutation are diffuse infiltrative Grade 2 astrocytomas (DIAs), while glial tumors with a Grade 2 phenotype but lacking 1p19q codeletion and IDH mutations are considered glioblastoma-like tumors or pre-glioblastomas

Mahmut OZDEN : 0000-0003-2441-0015
Orkhan MAMMADKHANLI : 0000-0003-3299-4196
Murat ZAIMOGLU : 0000-0001-5330-1251
Eyup BAYATLI : 0000-0001-6246-4247
Melih BOZKURT : 0000-0001-7433-081X

(pre-GBMs) with poor survival (3,23). Without a doubt, the patient's prognosis improves when the resection rates of glial tumors increase (7,22). Nonetheless, if the residue level is equal to or lower than 10% in postoperative early MR images, the prognosis is not changed substantially for oligodendrogliomas, whereas the prognosis is significantly influenced in diffuse LGGs (WHO Grade 2) and pre-GBMs (22,23). A second-look operation may be considered if more than 10% of the residual tumor exists in non-eloquent areas (23). Therefore, it is crucial to be able to differentiate these tumors intraoperatively. Hence, the potential of fluorescein in the differential staining of distinct types of WHO Grade 2 gliomas is of great interest, as it is crucial to reveal whether the fluorescein staining of the three subtypes of noncontrast-enhancing gliomas differs. This study determined whether fluorescein staining could facilitate distinguishing which of the three subgroups low-grade glioma (LGG) tumors belonged to during surgery because it can be challenging to predict the type of noncontrast-enhancing gliomas. We retrospectively compared the fluorescein staining characteristics of these three tumor types, which were confirmed by pathological and genetic studies. We hypothesized that DIA and pre-GBM might be more prone to absorb and retain fluorescein stains than oligodendrogliomas.

MATERIAL and METHODS

Patients between 18 and 76 years old who had never had neurological surgery before and had a T2/fluid attenuated inversion recovery (FLAIR)-hyperintense tumor, indicating a possible noncontrast-enhancing glioma as observed on preoperative MRI, were included in this study between July 2019 and September 2022. This study was approved by the Institutional Review Board (57, Memorial Bahcelievler Hospital). We received preoperative consent forms from all patients before their surgery. Patients with recurrent gliomas, brain stem tumors, postoperative pathology confirmed as glioblastoma or metastatic tumors, neurological diseases, or a history of previous brain surgery were excluded from this study. Clinical data were collected from patient records. Patients' intraoperative video recordings and preoperative magnetic resonance imaging (MRI) were analyzed for each patient. Besides routine histopathological analyses, IDH R132H mutation was determined immunohistochemically by clone H09 (Dianova) antibodies developed against mutant IDH protein. Fluorescence in situ hybridization analysis for the 1p19q codeletion was performed according to the method described by Kong et al. (13). In cases younger than 55 years old whose tumors were not stained with IDHR132H, other IDH1 and IDH2 mutations were investigated by sequencing, which revealed no mutations (data not shown). Histopathologically, patients were divided into WHO Grade 2 oligodendrogliomas, diffuse astrocytomas (IDH mutant, 1p19q negative tumors), and pre-GBMs (IDH wild type, 1p19q negative tumors). Resection margins were checked using control contrast-enhanced cranial MRI at the postoperative 24–72 h.

Surgical Protocol and Fluorescein Application

All patients included in the study underwent craniotomies and tumor resection surgeries. Neuronavigation systems (Brainlab AG, München, Germany; Medtronic, MN, USA) and intraoperative neurophysiological monitoring were performed in all procedures. Fluorescein sodium 10% (FLUOSINE 500 mg/5 ml, Pharm Argus, Istanbul) was administered intravenously at a dose of 5 mg/kg during the dural opening. Operating microscopes equipped with fluorescein filters were used in all cases (OPMI Pentero 900 with YELLOW560 fluorescence visualization kit, Carl Zeiss, Jena, Germany; M525 OH4, Leica Biosystems, Nussloch, Germany). The tumor resection target was determined according to the correlation of preoperative FLAIR and T2 images with navigation systems and the staining status with fluorescein. The shift of 5 mm after tumor resection was considered within normal limits in navigation systems. After the pathology reports of all patients were obtained, the surgical video recordings were reviewed, and the differences in brightness of fluorescein staining between different histopathology results were compared.

Fluorescein Staining

The brightness and intensity of the fluorescein stain of the tumor tissue could not be numerically classified. After the pathological results of the cases included in the study were obtained, the brightness degree of the tumors with fluorescein stain was retrospectively evaluated by the first and last authors independently and without knowing which tissue came from which patient. The brightness level was classified into three grades: intense, moderate, and mild. When inconsistency was found between the two authors in the cases, they were evaluated together, and a consensus was reached.

Statistical Analysis

Descriptive statistical methods (frequency, percentage) were used while evaluating the study data. An open-source statistics program, Jamovi, was used for the statistical analysis to reveal a general intragroup difference by employing Fisher exact test. Fisher–Freeman–Halton test was used to determine staining differences between different subtypes of LGGs. Statistical significance was accepted as $p<0.05$.

RESULTS

Fluorescein-guided resection was performed on 46 patients who had non-enhancing T2/FLAIR hyperintense lesions on MRI presumed LGGs. The general features of the patients in this study are summarized in Table I. Of the patients, 24 (52%) were women, and 22 (48%) were men. The patients were between the ages of 18 and 76 years (median, 39.5). Most patients, 19 in total, had 1p19q negative and IDH-1 positive and were considered to have DIA. Eighteen patients had 1p19q positive and IDH-1 positive, considered oligodendroglioma, and 9 patients with 1p19q negative and IDH-1 negative, considered pre-GBM. Thirty-six patients were admitted with headaches, nine seizures, and one focal neurological deficit. Table II lists the resection rate and staining density comparison according to the pathology results. According to the MR images obtained after the surgery, the tumor removal rates of the patients were as follows: In the group of oligodendroglioma patients (n=18), 13, 3, and 2 of cases underwent gross total, subtotal, and supramaximal resection, respectively. In

Table I: General Features of the Patients

Patient No	Age (years)	Gender	Localization	Symptom	1p19q	IDH-1
1	41	M	R. frontoparietal	Headache	Deleted	mt
2	57	M	L. frontal, R. temporal	Headache	Deleted	mt
3	32	F	R. frontal	Headache	Deleted	mt
4	30	M	R. parietal	Headache	Deleted	mt
5	40	F	L. frontal	Headache	Deleted	mt
6	48	F	R. parietal	Headache	Deleted	mt
7	42	M	L. frontotemporal	Headache	Deleted	mt
8	32	F	L. frontal	Headache	Deleted	mt
9	46	F	R. frontal	Focal Neurological Deficit	Deleted	mt
10	50	M	R. temporal	Seizure	Deleted	mt
11	34	F	R. frontal	Seizure	Deleted	mt
12	31	M	R. parietal	Headache	Deleted	mt
13	24	M	L. parietal	Headache	Deleted	mt
14	18	F	Occipital	Headache, cluster type	Deleted	mt
15	39	F	Occipital	Headache	Deleted	mt
16	63	M	L. cingulate and superior frontal	Headache	Deleted	mt
17	16	M	Occipital	Headache	Deleted	mt
18	62	M	R. frontal	Headache	Deleted	mt
19	37	F	L. frontotemporoparietal	Headache	Intact	mt
20	26	F	R. frontal	Headache	Intact	mt
21	51	F	L. frontoparietal	Headache	Intact	mt
22	44	M	L. frontotemporal	Headache	Intact	mt
23	21	M	L. frontal	Headache	Intact	mt
24	43	F	L. parietal	Headache	Intact	mt
25	31	F	L. frontal, R. temporal	Headache	Intact	mt
26	18	M	Occipital	Headache	Intact	mt
27	55	F	R. frontal	Headache	Intact	mt
28	29	F	L. frontal	Seizure	Intact	mt
29	23	F	L. insular	Seizure	Intact	mt
30	48	M	L. hippocampal	Seizure	Intact	mt
31	21	M	R. frontal	Headache	Intact	mt
32	35	F	L. frontoparietal	Headache	Intact	mt
33	40	M	L. sensorimotor	Headache	Intact	mt
34	59	M	L. angular gyrus	Headache	Intact	mt
35	53	F	L. precuneus	Headache	Intact	mt
36	34	M	L. temporal	Seizure	Intact	mt
37	76	M	L. frontal	Headache	Intact	mt
38	72	M	L. parietooccipital	Seizure	Intact	wild type
39	42	F	L. parietal	Headache	Intact	wild type
40	71	M	R. temporal	Headache	Intact	wild type
41	37	M	Insular	Headache	Intact	wild type
42	55	M	L. parietal	Headache	Intact	wild type
43	32	M	L. frontal	Seizure	Intact	wild type
44	28	F	L. frontal	Seizure	Intact	wild type
45	42	F	L. temporobasal	Headache	Intact	wild type
46	36	M	R. frontoparietal	Headache	Intact	wild type

M: *Male,* ***F:*** *Female,* ***R.:*** *Right,* ***L.:*** *Left,* ***mt:*** *mutant.*

Table II: Comparison of Resection Rate and Fluorescein-Staining Densities

Oligodendroglioma (n=18)		Pathology			p-value
		DIA (n=19)	Pre-GBM (n=9)		
Resection Rate	Gross Total	13 (72.2)	16 (84.2)	6 (66.7)	[a]***0.397***
	Subtotal	3 (16.7)	2 (10.5)	3 (33.3)	
	Supramaximal	2 (11.1)	1 (5.3)	0 (0)	

*[a]Fisher Freeman Halton Test; **p<0,01.* ***DIA:*** *Diffuse Infiltrative Astrocytoma,* ***Pre-GBM:*** *Pre-Glioblastoma*

Table III: Staining Densities of Different Subtypes of Low Grade Gliomas

	Staining Density			
Pathology	**Mild (1)**	**Moderate (2)**	**Intense (3)**	**Total**
Diffuse Astrocytoma	2	16	1	19
Oligodendroglioma	15	3	0	18
Pre-Glioblastoma	1	1	7	9
Total	18	20	8	46
Intergroup Difference, Fisher Exact Test. p<0.001				

the group of diffuse astrocytoma patients (n=19), 17, 1, and 1 of cases underwent gross total, subtotal and supramaximal resection, respectively. Lastly, in the pre-GBM patient group (n=9), 6 and 3 cases underwent gross total and subtotal resection, respectively (Table II). According to the pathology results, no statistically significant difference was found between the resection rate groups of the cases (p>0.05).

Neurological Status

One patient temporarily developed supplemental motor area syndrome but fully recovered on the third-month follow-up. One patient temporarily developed increasing dysphasia but fully recovered after steroid treatment.

Fluorescence Imaging Results

Figures 1 and 2 depict fluorescein staining of varying subtypes of LGGs; their corresponding inscriptions describe the stunning staining differences. Table III summarizes fluorescein staining densities of different subtypes of LGGs. Fisher's exact test revealed a highly significant difference (p=0.001; p<0.01) between different subtypes of LGGs in terms of fluorescein staining. With the fluorescein administration, LGGs (1p19q codel-negative, IDH-mutant type) and pre-GBMs (1p19q codel-negative, IDH wild type) were stained with fluorophore dye, and their limits were discernible under the surgical microscope's fluorescence filter. Conversely, low-grade oligodendrogliomas (1p19q codel-positive and IDH-mutant type) were barely stained with fluorescein, and their limits cannot be distinguished. Oligodendrogliomas were more likely to accumulate in the mild (1) staining group than DIAs and pre-GBMs (p<0.001). DIAs were more likely to accumulate in the moderate (2) staining group than in oligodendrogliomas and pre-GBMs (p<0.001). Finally, pre-GBMs were more likely to accumulate in the intense (3) staining group than oligodendrogliomas and DIAs (p<0.001).

DISCUSSION

Low-grade glial brain tumors are slow-growing malignant tumors that are, unfortunately, not curable. Even with treatment, it is impossible to cure them completely, and they will occur again at a certain time. The main goal of surgery for glioma treatment is to be as "complete" as possible, which can be challenging to achieve because gliomas are infiltrative and often located in eloquent areas (20). Gross total resection (GTR) is the most significant factor in increasing overall survival in LGGs because the increased resection ratio both prolongs the time of relapse and significantly increases the efficacy of systemic treatments, such as radiotherapy and chemotherapy (7). Over time, different technologies have been developed in the field of neurosurgery that can increase the chances of being able to remove as much of an LGG tumor as possible while also providing maximum safety for the patient. These methods can be classified into three main categories: neuronavigation systems, intraoperative imaging systems, such as USG, CT, and MRI, and tumor staining methods, such as fluorescein and 5-ALA (11). Studies have shown that the increase in the resection rate in low-grade tumors with 1p19q codeletion and IDH mutation called oligodendrogliomas does not lead significant improvements in survival compared with the DIAs and pre-GBM tumors (23). Therefore, the postoperative morbidity rates will decrease if a surgical tool can distinguish between these three subgroups of tumors intraoperatively.

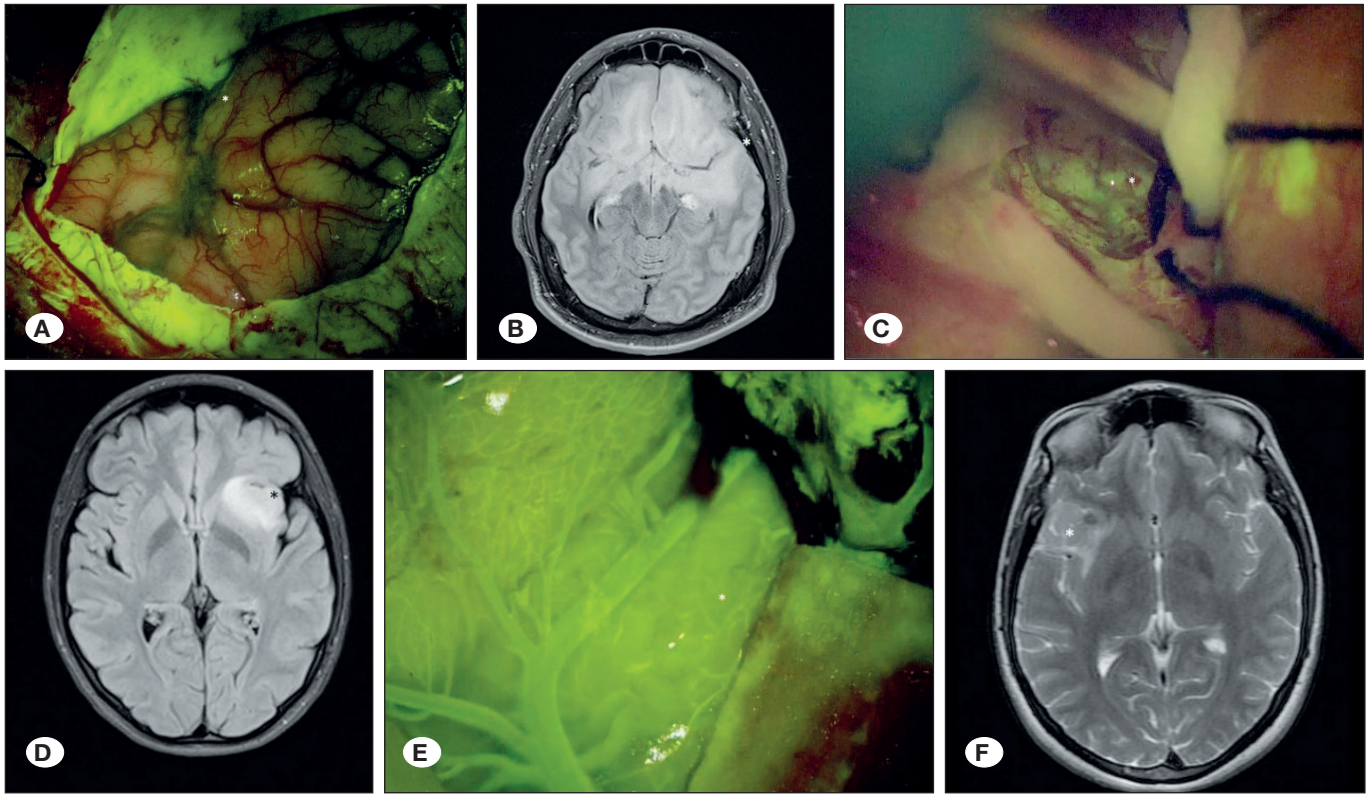

Figure 1: A) Fluorescein staining intensity of an oligodendroglioma. **B)** Preoperative T2-TSE-dark-fluid-postcontrast MRI of the oligodendroglioma patient. **C)** Fluorescein staining intensity of a DIA. **D)** Preoperative T2-TSE-dark-fluid-postcontrast MRI of the DIA patient. **E)** Fluorescein staining intensity of a pre-GBM. **F)** Preoperative T2 MRI of the patient with pre-GBM. (*) Asterisk marks the same sectional area of the tumors.

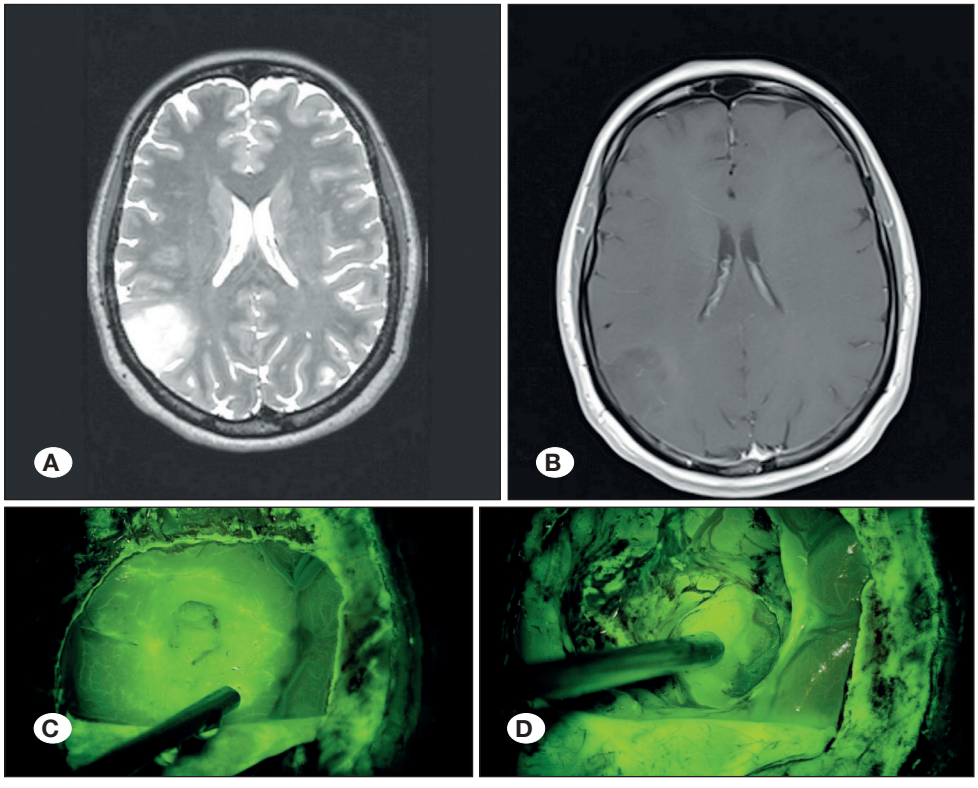

Figure 2: A) T2-weighted MRI image of a pre-GBM patient.
B) T1-contrast-enhanced MRI image of the same patient.
C,D) Intraoperative fluorescein staining highlighting the tumor.

Devices available during surgery are limited in their ability to determine whether the tumor is an oligodendroglioma, diffuse astrocytoma, or pre-GBM, and CT or MR can only help achieve this confirmation if there is calcification (21). Although FLAIR (Fluid Attenuated Inversion Recovery), MRS (Magnetic Resonance Spectroscopy), and perfusion MRI (Magnetic Resonance Imaging) techniques have improved the ability to assess tumor histopathologies preoperatively, their success in distinguishing LGG subtypes is limited. Many MR-based studies have been conducted to differentiate low-grade glial tumors before surgery. Diffuse LGGs not only infiltrate the neighboring normal-appearing cerebral parenchyma but also detect tumor cells up to 20 mm beyond the area of MRI abnormal regions, even in well-demarcated gliomas (25). Furthermore, the absence of enhancement of a glial tumor with contrast on MRI does not necessarily mean it is a low-grade tumor. In an early study published in 1998, Ginsberg et al. retrospectively compared the preoperative MRI features and postoperative histopathological results of 40 adult patients with supratentorial glial tumors (8). The researchers found that the lack of contrast enhancement in the tumors was not necessarily indicative of a low-grade tumor; 40% of the tumors examined were high-grade gliomas. Therefore, they suggested that a more aggressive surgical treatment should always be considered (8).

Moore and colleagues first tried the idea of detecting tumors in neurosurgery using fluorescein staining in Minnesota in 1948, and of the 46 evaluated patients, 44 were successfully diagnosed with a brain tumor (14). When fluorescein is injected intravenously into a patient with a glial tumor, the tumor will absorb the 560 nm of fluorescein light source and then emit a bright green color. With the help of filters on advanced microscope systems, shining parts of the brain can be easily distinguished from non-shining areas so that more tumor resection can be achieved. Many studies have demonstrated the absorption and brightness of fluorescence in contrast-enhancing HGGs and metastatic lesions, and the pathophysiology has been revealed in a definite and comprehensive manner (1,2,12,24). Fluorescein sodium, having a molecular weight of 376 Da, is one of the smallest molecules to define a BBB disruption, while gadolinium utilized as an MRI-contrast agent harbors a higher molecular weight of 552 Da. Thus, fluorescein may act more sensitively to define the neurovascular unit disruption caused by glial tumor cells (16).

In our country (Turkey), the clinical study group of Kiris et al. reported their experience with fluorescein staining in brain tumors since 2016 (9,10). In their first study, they used 200 mg (2–4 mg/kg) of fluorescein in 28 patients (30 surgeries consisted of 23 high-grade gliomas and 7 metastatic tumors) after anesthesia induction (10). The employment of the YELLOW 560-nm filter was regarded as helpful if the distinction of the healthy brain tissue, and the fluorescein-stained tumor tissue was clear; otherwise, it was defined as not helpful. Fluorescein filter was helpful for tumor demarcation in 29 of 30 operations (97%) and in 23 of these 29 operations (79%); total resection was capable with no adverse effects, suggesting the safety and feasibility of this method (10). In a relatively more recent investigation of the same clinical study group, the experience with fluorescein administration was reported for pediatric brain tumor surgery (9). Among 23 patients (mean age: 9.4 years) with 7 tectal plate/brain stem gliomas, 6 supratentorial, 4 intraventricular, 2 pineal and 2 infratentorial tumors, 1 clivus tumor, and 1 malignancy with infratentorial and supratentorial extensions were analyzed. Fluorescein was helpful for tumor demarcation in 20 patients (87%). In 11 of these 20 surgeries (55%), a total resection was capable regardless of lesion pathology, while subtotal resection was possible in the remaining 9 patients (45%) with no adverse reactions, thus suggesting the utility and safety of this procedure (9).

According to our observations, the degree of fluorescein staining was considerably proportional to the grade of the tumor. It was initially thought that fluorescein would be retained if there was a disruption in the blood-brain barrier. Therefore, it can be expected that non-enhancing LGG should not show staining with fluorescein. However, it is frequently observed in intraoperative observations that low-grade glial tumors are stained with fluorescein, even if MRI does not reveal contrast enhancement. Indeed, in their 32 cases in 2016, Neira et al. concluded in their intraoperative fluorescein staining evaluation of patients with GBM that even non-contrast-enhanced areas in MRI showed fluorescence staining and extended the margins of tumor removal to the area of fluorescein staining (15). Bowden and colleagues checked the histopathological characteristics of both fluorescent and nonfluorescent neural tissues taken from NE gliomas (5). They could assess which parts of the tumor were more malignant using fluorescein guidance (5). Schebesch reported five patients with MRI non-enhancing but 18F-fluoroethyl tyrosine positron-emission tomography (FET-PET) positive gliomas who underwent fluorescein (5 mg/kg)-guided surgery (19). They found that the areas of the target lesions that showed fluorescein staining were similar to those that tested positive for FET-PET. In addition, their study found that, regardless of the final tumor grades, fluorescein was very helpful in determining the lesions and lesion border regions (19).

In addition to these three studies, the fluorescein uptake properties of LGGs were evaluated in detail related to the fluorescein uptake of LGGs to the literature to get a more comprehensive understanding (6,16,17). However, a study comparing the fluorescein staining degrees of subtypes of LGGs has not been found in the literature. In diffuse gliomas, adequate tumor tissue sampling representing the lesions' highest malignant potential is essential for their surgical management as the likely existence of more proliferative and hypercellular microfoci within LGGs is associated with poor overall survival (5,18). Both high- and low-grade pathological components inside the same tumor lead to a significant risk of underdiagnosing neoplastic tissue. Incapability to determine these areas containing both low- and high-grade tumor regions may cause clinical undergrading and insufficient treatment (5). Importantly, tissue specimens obtained from fluorescent regions were more likely to exert glial tumor tissues (with a 95% positive predictive value for the existence of a tumor diagnostic tissue), cytological atypia, greater total cellularity, and cell proliferation (5). These results agree with our study's findings, suggesting an association between

increased biological aggression and fluorescein uptake in LGGs. In our study, the least fluorescein uptake was observed in oligodendrogliomas, and this is the tumor type with the most benign biological behavior among the three different tumors we examined. Among the three tumor types studied, DIAs exhibit a biological behavior between oligodendroglioma and pre-GBMs, and these tumors showed moderate staining intensity. Finally, among the three different LGG types examined, pre-GBMs had the highest potential for malignant behavior, and the most intense fluorescein staining was observed in these tumors. From these results, it can be hypothesized that there is a positive correlation between increased aggressivity inherent in LGGs and blood-brain barrier disruption; therefore, fluorescein uptake is a guiding indicator for the surgeon, at least relatively. We hypothesize that fluorescein helps identify and distinguish the subtypes of LGGs. Therefore, we would suggest that fluorescein is a useful diagnostic tool to have during surgery for LGGs. That is, a more aggressive resection can be forced by estimating which subtype of LGG is based on the degree of staining, thus contributing to prolonging the patient's survival time. In addition, this will reduce the possibility of second-look surgery.

Limitations of the Study

One of the main drawbacks of this study is that the degree of staining using fluorescein cannot be accurately measured with a numerical value. The intensity of the fluorescent staining can be interpreted as mild, moderate, and intense, according to the personal perceptions of the authors. Two different microscopes were used for our operations and some cases were operated with one, and the others were operated with another microscope. These filter differences can be considered a shortcoming of the study; however, we consistently observed that the staining characteristics, which differ according to the biological aggressiveness of tumors were in the same direction independent of the microscope brands (data not shown). Another limitation is that this is a retrospective study, which included a relatively small number of patients. We have not randomized the patients even though the baseline characteristics were similar in the three groups. According to the immunohistochemistry and genetic results, the diagnosis of diffuse astrocytoma or pre-GBM is controversial in cases where no 1p19q codeletion is detected. Although the histopathological diagnosis changes in the presence of additional molecular indicators, such as EGFR and hTERT mutations, we made our subclassification according to the IDH mutation status. We defined the IDH wild-type tumors as pre-GBM and the IDH-mutant one as diffuse astrocytoma, and we detected a gradual difference in staining patterns between the three LGGs. As these results are encouraging, the focus of this work merits more extensive prospective randomized trials investigating the usage of fluorescein staining in LGGs.

CONCLUSION

A difficulty may be encountered in determining tumor margins in LGG surgery. Extent resection of low-grade tumors is increased using anatomical landmarks and intraoperative imaging methods. When used together, preoperative MRI studies, intraoperative frozen studies, and fluorescein staining have complementary properties in identifying LGG subtypes. Our data in this retrospective analysis of patients confirmed that using intravenous fluorescein is helpful during the resection of LGGs. In our study, we observed that there was fluorophore uptake with the use of intraoperative fluorescein in low-grade glial masses. Additionally, it was shown that it was related to a high tumor resection rate, as demonstrated with the early MRI after 24–72 h of surgery. In conclusion, we propose that fluorescein is a practical intraoperative guide to help the different subtypes of noncontrast enhancing tumors intraoperatively.

AUTHORSHIP CONTRIBUTION

Study conception and design: MB
Data collection: MO, OM
Analysis and interpretation of results: MO, OM
Draft manuscript preparation: MO, OM, MB
Critical revision of the article: MB, MZ, EB
Other (study supervision, fundings, materials, etc...): MB, MZ, EB
All authors (MO, OM, MZ, EB, MB) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Acerbi F, Broggi M, Eoli M, Anghileri E, Cavallo C, Boffano C, Cordella R, Cuppini L, Pollo B, Schiariti M, Visintini S, Orsi C, La Corte E, Broggi G, Ferroli P: Is fluorescein-guided technique able to help in resection of high-grade gliomas? Neurosurg Focus 36:E5, 2014
2. Acerbi F, Broggi M, Schebesch KM, Höhne J, Cavallo C, De Laurentis C, Eoli M, Anghileri E, Servida M, Boffano C, Pollo B, Schiariti M, Visintini S, Montomoli C, Bosio L, La Corte E, Broggi G, Brawanski A, Ferroli P: Fluorescein-guided surgery for resection of high-grade gliomas: A multicentric prospective Phase II Study (FLUOGLIO). Clin Cancer Res 24:52-61, 2018
3. Aibaidula A, Chan AK, Shi Z, Li Y, Zhang R, Yang R, Li KK, Chung NY, Yao Y, Zhou L, Wu J, Chen H, Ng HK: Adult IDH wild-type lower-grade gliomas should be further stratified. Neuro Oncol 19:1327-1337, 2017
4. Bai J, Varghese J, Jain R: Adult glioma WHO classification update, genomics, and imaging: What the radiologists need to know. Top Magn Reson Imaging 29:71-82, 2020
5. Bowden SG, Neira JA, Gill BJA, Ung TH, Englander ZK, Zanazzi G, Chang PD, Samanamud J, Grinband J, Sheth SA, McKhann GM 2nd, Sisti MB, Canoll P, D'Amico RS, Bruce JN: Sodium fluorescein facilitates guided sampling of diagnostic tumor tissue in nonenhancing gliomas. Neurosurgery 82(5):719-727, 2018
6. Choi J, Kim SH, Ahn SS, Choi HJ, Yoon HI, Cho JH, Roh TH, Kang SG, Chang JH, Suh CO: Extent of resection and molecular pathologic subtype are potent prognostic factors of adult WHO grade II glioma. Sci Rep 10:2086, 2020

7. Falco J, Cavallo C, Vetrano IG, de Laurentis C, Siozos L, Schiariti M, Broggi M, Ferroli P, Acerbi F: Fluorescein application in cranial and spinal tumors enhancing at preoperative MRI and operated with a dedicated filter on the surgical microscope: Preliminary results in 279 patients enrolled in the FLUOCERTUM prospective study. Front Surg 6:49, 2019
8. Ginsberg LE, Fuller GN, Hashmi M, Leeds NE, Schomer DF: The significance of lack of MR contrast enhancement of supratentorial brain tumors in adults: Histopathological evaluation of a series. Surg Neurol 49:436-440, 1998
9. Goker B, Kiris T: Sodium fluorescein-guided brain tumor surgery under the YELLOW-560-nm surgical microscope filter in pediatric age group: Feasibility and preliminary results. Childs Nerv Syst 35(3):429-435, 2019
10. Hamamcioglu MK, Akcakaya MO, Goker B, Kasimcan MO, Kiris T: The use of the YELLOW 560 nm surgical microscope filter for sodium fluorescein-guided resection of brain tumors: Our preliminary results in a series of 28 patients. Clin Neurol Neurosurg 143:39-45, 2016
11. Ius T, Mazzucchi E, Tomasino B, Pauletto G, Sabatino G, Della Pepa GM, La Rocca G, Battistella C, Olivi A, Skrap M: Multimodal integrated approaches in low grade glioma surgery. Sci Rep 11:9964, 2021
12. Kofoed MS, Pedersen CB, Schulz MK, Kristensen BW, Hansen RW, Markovic L, Halle B, Poulsen FR: Fluorescein-guided resection of cerebral metastases is associated with greater tumor resection. Acta Neurochir (Wien) 164:451-457, 2022
13. Kong PL, Cheah PL, Mun KS, Chiew SF, Lau TP, Koh CC, Teoh KH, Nazarina AR, Looi LM: FISHing for 1p19q codel in oligodendroglioma. Malays J Pathol 42:369-376, 2020
14. Moore GE, Peyton WT, French LA, Walker WW: The clinical use of fluorescein in neurosurgery; the localisation of brain tumors. J Neurosurg 5:392-398, 1948
15. Neira JA, Ung TH, Sims JS, Malone HR, Chow DS, Samanamud JL, Zanazzi GJ, Guo X, Bowden SG, Zhao B, Sheth SA, McKhann GM 2nd, Sisti MB, Canoll P, D'Amico RS, Bruce JN: Aggressive resection at the infiltrative margins of glioblastoma facilitated by intraoperative fluorescein guidance. J Neurosurg 127:111-122, 2017
16. Nevzati E, Chatain GP, Hoffman J, Kleinschmidt-DeMasters BK, Lillehei KO, Ormond DR: Reliability of fluorescein-assisted stereotactic brain biopsies in predicting conclusive tissue diagnosis. Acta Neurochir (Wien) 162:1941-1947, 2020
17. Patel SH, Bansal AG, Young EB, Batchala PP, Patrie JT, Lopes MB, Jain R, Fadul CE, Schiff D: Extent of surgical resection in lower-grade gliomas: Differential impact based on molecular subtype. AJNR Am J Neuroradiol 40:1149-1155, 2019
18. Pedeutour-Braccini Z, Burel-Vandenbos F, Gozé C, Roger C, Bazin A, Costes-Martineau V, Duffau H, Rigau V: Microfoci of malignant progression in diffuse low-grade gliomas: Towards the creation of an intermediate grade in glioma classification? Virchows Arch 466:433-444, 2015
19. Schebesch KM, Brawanski A, Doenitz C, Rosengarth K, Proescholdt M, Riemenschneider MJ, Grosse J, Hellwig D, Höhne J: Fluorescence-guidance in non-Gadolinium enhancing, but FET-PET positive gliomas. Clin Neurol Neurosurg 172:177-182, 2018
20. Senders JT, Muskens IS, Schnoor R, Karhade AV, Cote DJ, Smith TR, Broekman ML: Agents for fluorescence-guided glioma surgery: A systematic review of preclinical and clinical results. Acta Neurochir (Wien) 159:151-167, 2017
21. Smits M: Imaging of oligodendroglioma. Br J Radiol 89: 20150857, 2016
22. Tom MC, Cahill DP, Buckner JC, Dietrich J, Parsons MW, Yu JS: Management for different glioma subtypes: Are all low-grade gliomas created equal? Am Soc Clin Oncol Educ Book 39:133-145, 2019
23. Wijnenga MMJ, French PJ, Dubbink HJ, Dinjens WNM, Atmodimedjo PN, Kros JM, Smits M, Gahrmann R, Rutten GJ, Verheul JB, Fleischeuer R, Dirven CMF, Vincent AJPE, van den Bent MJ: The impact of surgery in molecularly defined low-grade glioma: An integrated clinical, radiological, and molecular analysis. Neuro Oncol 20:103-112, 2018
24. Xiang Y, Zhu XP, Zhao JN, Huang GH, Tang JH, Chen HR, Du L, Zhang D, Tang XF, Yang H, Lv SQ: Blood-brain barrier disruption, sodium fluorescein, and fluorescence-guided surgery of gliomas. Br J Neurosurg 32:141-148, 2018
25. Yordanova YN, Duffau H: Supratotal resection of diffuse gliomas - an overview of its multifaceted implications. Neurochirurgie 63:243-249, 2017