Cross-sectional Study

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Safety, efficacy, and side effects of sodium fluorescein-aided resection of glioblastoma: a quasi-experimental study

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Background: The use of fluorescein sodium (FS) as a surgical adjunct in glioblastoma resection has shown promise in improving tumor visualization and resection outcomes. This study aimed to evaluate the safety, efficacy, and side effects of FS-aided resection in patients with glioblastoma.

Methods: This is a prospective, single-center cohort study conducted at Ibrahim Cardiac Hospital and Research Institute from September 2021 to November 2023. Twelve patients with histologically confirmed glioblastoma underwent FS-guided resection. All participants received an intravenous dose of FS (5 mg/kg body weight) ~30 min before surgery. The study follows a quasi-experimental design, focusing on the outcomes of FS-aided surgery without a control group. Patients were selected based on specific inclusion and exclusion criteria, and all surgeries were performed by a single experienced neurosurgeon. The extent of tumor resection was classified as gross total resection (GTR), near-total resection (NTR), or partial resection (PR).

Results: Gross total resection (GTR) was achieved in 66.6% of patients, near total resection (NTR) in 16.6%, and subtotal resection (STR) in 16.6%. No significant adverse effects were observed except for a single case of postoperative seizure, which was managed without long-term consequences. All patients showed normal liver and kidney function tests postoperatively. The low-dose FS protocol demonstrated both a high rate of GTR and a favorable safety profile, with only minor, transient side effects such as temporary yellow discoloration of the skin, sclera, and urine. No severe or long-term complications related to FS were observed during the follow-up period, which had a median duration of 13.4 months.

Conclusion: FS appears to be a safe and effective aid in glioblastoma resection, achieving high rates of GTR with minimal side effects. The findings suggest that FS, particularly at a low dose, is a viable, cost-effective alternative to other fluorescent markers, especially in settings where resource constraints may limit the use of more expensive options like 5-ALA.

Keywords: 5-aminolevulinic acid (5-ALA), cost-effectiveness, fluorescein sodium (FS), glioblastoma, gross total resection, safety

Introduction

Glioblastoma multiforme (GBM) affects 0.59 to 5 people out of every 100 000, and it is becoming more common in many countries. This increase has multiple causes, some of which are an ageing population, overdiagnosis, ionizing radiation, and air pollution[1]. The prognosis for patients with high-grade glioblastoma (HGG) depends on a number of factors, including age, the location of the tumors, radiological characteristics, and the possibility of doing adjuvant therapy during the postoperative period. Previous research indicates the cumulative 5-year survival of patients is 5.8% (0.01–29.1%) and the median overall survival (MOS) is 13.5 months (2.3–29.6)[2,3].

HIGHLIGHTS

- Several fluorescent biomarkers improve intraoperative identification of residual tumors; among these, 5-aminolevulinic acid (5ALA) and fluorescein sodium (FS) have emerged to play a central role in glioma surgery.
- This study aimed to analyze the safety, efficacy, and side effects of using fluorescein sodium (FS)-aided resection of glioblastoma.
- We have collected information from 12 surgically treated patients with glioblastomas at a research institute and found that FS seems to be safe and effective in the resection of glioblastomas, allowing a high rate of GTR.

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There is a substantial link between overall survival and the degree of GBM clearance. When adjuvant radiation and chemotherapy are given in conjunction with a resection, the volume would be greater than 98%, and the greatest effect on survival is observed[4,5]. However, total tumor excision is difficult due to the tumor's close resemblance to the surrounding brain parenchyma under the operating microscope[6]. In order to maximize the extent of resection, FS and 5-ALA have shown the greatest pro mise in HGG surgery among the fluorescent biomarkers that have been studied to enhance intraoperative identification of malig nant tissue from the normal brain[7–9].

5-ALA is highly sensitive and specific for tumoral tissues; however, there are a few barriers that prevent its broad application in the excision of gliomas. The fact that it is expensive, that it must be taken orally a few hours prior to the induction of anesthesia, and that there is a significant chance of skin sensitization 24 h following the procedure[7]. For these limitations, there has been an increasing trend towards the use of FS in HGG surgery[7,10].

The first application of FS in brain tumor surgery was reported by Moore *et al.* in 1947[11]. This generates fluorescent radiation with a wavelength range of 540–690 nm when stimulated by light with a wavelength in the 460–500 nm range. FS appears to play a role as a marker for regions where the blood brain barrier (BBB) is impaired, such as in high-grade astrocytomas, as it does not collect preferentially in astrocytoma cells but rather in extracellular tumor spaces[7]. In ophthalmic surgery, it has been widely and safely utilized[12]. Fluorescein is an inexpensive, nearly side-effect-free intravenous injection given right before glioma exci sion. It is usually visible to the naked eye at a high dosage (20 mg/kg body weight), and at a lower dosage, it is observable under the yellow 560 nm surgical microscope filter, allowing better tissue discrimination with more natural colors[7,10,12].

FS has been widely used in various medical fields for many years, particularly in ophthalmology. However, its use in brain tumor resection, is less well-established. Fluorescein sodium was the first agent used for neurosurgical imaging in the neurosurgery field based on its previous success in gastroenterological CLE imaging. Since then, it has been adopted and has achieved US Food and Drug Administration (FDA) approval for use in neurosurgery. FS has peak excitation at 494 nm and peak emission at 521 nm. Previous studies identified the timing for FS administration at the induction of anesthesia with a dose of 2–5 mg/kg. This timing and dosage have been used in a number of studies and widely used in Europe and the United States[13,14]. The timing of FS administration is critical to ensure optimal fluorescence during the surgical procedure. The short half-life of FS suggests that its fluorescent effect may diminish over time, the practical need for readministration depends on the duration of the surgery and the persistence of adequate fluorescence. Studies have shown that the fluorescence of FS remains sufficient for tumor visualization for several hours after administration. However, in exceptionally long procedures, a second dose of FS could be considered to maintain optimal visualization[13].

In this study, we have analyzed the information of 12 patients who were operated on for glioblastomas at our institute. Our aim is to demonstrate the effectiveness, safety, and side effects of fluorescence-guided resection in glioblastoma surgery. We focus on histology, tumor removal rate, preoperative and postoperative clinical parameters, and adverse effects.

Materials and methods

Study design

This was a prospective quasi-experimental study aimed to evaluate the safety, efficacy, and side effects of using fluorescein sodium (FS)-aided resection in patients undergoing surgery for glioblastoma. Our work has been reported in line with the strengthening the reporting of cohort, cross-sectional, and case–control studies in surgery (STROCSS) criteria. In our center, each patient suspected radiologically as high-grade glioma underwent FS-guided resection, so no control group was chosen for the statistical analysis. While our study follows a quasi-experimental design, it does not involve the random assignment of subjects to different treatment groups. Instead, all participants in this study received the same intervention—fluorescein sodium (FS)-aided resection of glioblastoma—without the inclusion of a control group undergoing standard resection without FS. Our study design is more akin to a single-arm study, where the focus is on evaluating the outcomes of a specific intervention within a defined group of patients, rather than comparing outcomes between different groups[15].

Participants

Twelve patients (nine males and three females) who underwent surgical treatment for glioblastomas at our institute between September 2021 and November 2023 were included in the study. Inclusion criteria encompassed patients aged 18 and 90 years with histologically proven cases of glioblastoma and the location of the tumor allowed a complete surgical resection of the enhanced area. Exclusion criteria consisted of recurrent cases, recent major surgery within 3 months; a history of severe reactions to contrast agents; pregnancy; severe heart, liver, or kidney disease; recent acute ischemic stroke; specific neural tumor locations such as corpus callosum, basal ganglia, brain stem, posterior cranial fossa; preoperative Karnofsky Performance Status (KPS) score <50; and history of non-neural malignant tumors.

Ethical considerations

Ethical approval was obtained from the local Ethical Approval Committee Ibrahim Cardiac Hospital and Research Institute (Ref: ICHRI/Research/ERC/2021/03). Each patient was informed about risks connected with the study and informed written consent was obtained from each of them.

Procedure

Prior to the skin incision, each patient received 5 mg/kg body weight of FS intravenously via a central venous line following the induction of anesthesia. Vital signs were monitored for 15 min postadministration for any adverse effects such as blood pressure changes, seizures, allergic reactions, or anaphylaxis. Under white light, no fluorescent effect was observed; however, under the yellow 560 nm filter of the OPMI Pentero 800 surgical microscope, the fluorescent dye was visible (Carl Zeiss Meditec)[16]. All procedures were performed by the same senior neurosurgeon to ensure consistency in assessment. This allowed a distinction between cases in which fluorescence was judged 'helpful' and cases in which it was considered 'not helpful', based on a clear intraoperative distinction between the normal brain and the tumor tissue. The strategy to avoid the unintended removal of

false-positive fluorescence due to manually-induced additional BBB disruption is to place a piece of cotton onto the non-fluorescent surgical field immediately after the fluorescent tumor tissue has been removed; this way, reresection can be prevented. Neuro-navigation based on T1-weighted gadolinium-enhancement MRI was used for tumor localization, mostly for the localization of close, and eloquent areas.

The term 'helpful' here is used to describe the effectiveness of FS in enhancing the visualization of glioblastoma tumor margins during resection. Specific intraoperative criteria include the ability of FS to clearly delineate the tumor boundaries from the surrounding normal brain tissue, allowing for more precise and complete tumor removal. The presence of distinct fluorescence in the tumor region viewed through the YELLOW 560 nm surgical filter, was considered a key indicator of 'helpfulness'. FS was deemed 'helpful' when it provided clear differentiation between fluorescent tumor tissue and nonfluorescent normal tissue, enabling the surgeon to achieve maximal resection while minimizing the risk of damaging healthy brain structures. Quantitatively, the extent of tumor resection was categorized into three levels: gross total resection (GTR), near-total resection (NTR), and partial resection (PR). GTR was defined as the absence of visible tumor tissue under the surgical microscope and on postoperative imaging, while NTR and PR were defined as the removal of more than 95% and less than 95% of the tumor, respectively, based on the assessment of the resection cavity. Postoperative noncontrast CT scans were used to evaluate the resection extent, with GTR being confirmed when no residual tumor was detectable on imaging.

Assessment

Clinical assessment

Patients were admitted to the neurosurgical ICU postoperatively. General physical performance was evaluated using the Karnofsky Performance Status (KPS) scale. A preoperative clinical evaluation was performed at admission to the neurosurgical unit. A second evaluation was conducted during the postoperative course, on discharge, and at the first outpatient clinic visit, 2 weeks later. Surgery was followed by radiotherapy with concomitant and adjuvant temozolomide in some cases, according to the Stupp protocol[17].

Radiological assessment

Preoperative MRI studies were conducted within one week before surgery, with postoperative noncontrast CT scan of the brain performed within hours after surgery (Fig. 1). The extent of tumor resection was categorized as gross total resection (GTR), near-total resection (NTR), or partial resection (PR) based on postoperative noncontrast CT scans. GTR was defined as resection where no residual tumor is visible, NTR was defined as nearly total ($>95\%$), and PR was defined as subtotal resection ($<95\%$) based on a postoperative noncontrast CT scan of the brain at the first postoperative day, verified by the radiologist by calculating the Hounsfield unit of different tissues at the resection bed.

Histological examination

Histological analyses were performed according to standard procedures, with confirmation through immunohistochemistry. Tumor classification followed the current WHO classification of central nervous system tumors[18].

Monitoring patients

Monitoring of adverse effects associated with the use of FS was conducted rigorously, following a standardized protocol designed to detect both immediate and late-onset complications. All patients were closely monitored in the neurosurgical ICU postoperatively, with continuous monitoring of vital signs for at least 24 h following surgery. This included regular assessments of blood pressure, heart rate, oxygen saturation, and neurological status to detect any acute adverse reactions such as seizures, allergic reactions, or hemodynamic instability. In addition to immediate postoperative monitoring, patients underwent scheduled follow-up visits at 2 weeks, 1 month, 3 months, and every 3 months thereafter, where clinical evaluations were performed to identify any delayed adverse effects. Liver and kidney function tests were routinely conducted preoperatively and repeated during the follow-up period to ensure that FS did not induce any late-onset organ dysfunction. The study also included imaging follow-up with noncontrast CT scans immediately postoperatively and MRI scans as clinically indicated, to assess for any complications such as postoperative hemorrhage, infection, or unexpected tumor recurrence.

Justification for the use of noncontrast CT scans

Noncontrast CT scans were chosen for the postoperative evaluation of tumor resection primarily due to their availability, speed, and reduced cost compared to gadolinium-enhanced MRI. In our single-center setting, noncontrast CT scans are routinely available and provide a quick assessment of postoperative conditions, particularly in identifying complications such as hemorrhage or edema immediately after surgery. Additionally, the use of noncontrast CT avoids the potential risks associated with gadolinium contrast agents, such as nephrogenic systemic fibrosis in patients with impaired renal function. However, we acknowledge that gadolinium-enhanced MRI is the gold standard for detecting residual tumor tissue due to its superior sensitivity and specificity for identifying residual glioma. The limitations of using noncontrast CT in our study are noted, including its reduced ability to differentiate between residual tumor tissue and post-surgical changes such as blood products or edema.

Methods for calculating tumor resection percentage

The extent of tumor resection was calculated based on postoperative noncontrast CT scans. The assessment was conducted by measuring the dimensions of the resection cavity on serial CT slices and comparing these measurements to preoperative imaging data to estimate the volume of the residual tumor. The percentage of tumor resection was determined using the following formula:

$$\text{Percentage of Tumor Resection} = \left[1-(\text{Volume of Residual Tumor}/\text{Preoperative Tumor Volume})\right] \times 100$$

No specific software tools were employed for volumetric analysis; instead, a manual estimation method was used based on radiological assessment by experienced neuroradiologists. We acknowledge that this method has its limitations in terms of accuracy and reproducibility.

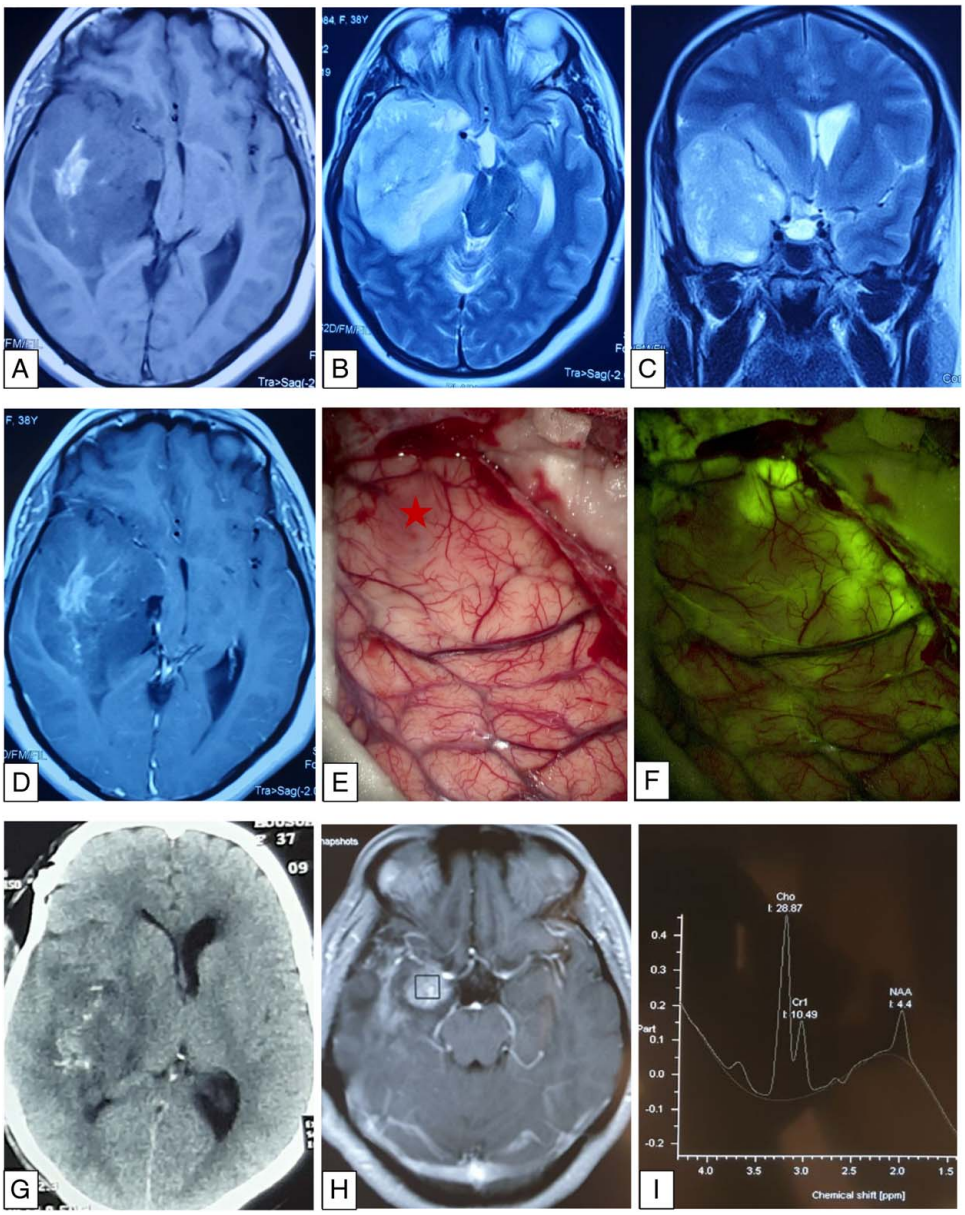

Figure 1. MRI of the brain, T1-weighted axial section demonstrated a well-defined 7×6 cm, predominantly hypointense lesion located in the right temporal lobe. Mass effect is evident by compression on cerebral peduncle with moderate midline shifting (A); the lesion becomes heterogeneously hyperintense in T2WI with marginal flow void of right M1 segment of the middle cerebral artery (MCA). There is significant compression and upward displacement of both M2 segments of MCA (B and C). After administration of contrast, there is mild heterogeneous contrast enhancement (D). Intraoperative view under normal white xenon-light illumination demonstrated an ill-defined greyish red area (marked by 5 point star), comparing the surrounding brain parenchyma (E) and at the beginning of tumor removal under the YELLOW 560 nm filter, fluorescent enhanced area become well visualized (F). A postoperative CT scan of the brain showed no residual tumor with significant reduction of mass effect (G). Follow-up MRI of the similar patient done after 18 months of receiving adjuvant chemoradiation under the Stupp protocol demonstrated a small contrast-enhanced area in the resected zone (H), which was compatible with postradiation pseudoprogression in magnetic resonance spectroscopy (I).

Results

At neuropathological analysis, all patients (Table 1) were confirmed as cases of glioblastoma with Ki 67 labeling index with MG/MT methylation status. The mean age of the participants was 57 years, ranging from 38 to 73 years (Table 2). The median preoperative Karnofsky Performance Status (KPS) score was 60 (mean 62.5) with a range of 50–80. The median postoperative KPS score was 80 and mean was 81.7 (range: 60–100). Comparing preoperative and postoperative scores, the postoperative scores were found to be higher in all the patients. Table 2 has summarized demographic characteristics, tumor locations, presentation, and preoperative KPS.

Follow-up

The overall median follow-up period was 13.4 months (range:

Table 1

Summary of characteristics of confirmed cases of glioblastoma.

S/N	Age/sex	Location	Presentation	KPS (Preoperative)	Status of resection	Postoperative complications	KPS (Postoperative-after 1 month)	Adjuvant chemoradiation	Overall survival
01	38/F	Right temporal	AMS, Hemiparesis	80	GTR	None	100	External beam radiation-50Gy, SBRT based cyberknife-10Gy, Tab Temozolomide	23 months (alive)
02	70/M	Right parieto-occipital	Hemiparesis	50	GTR	Transient aggravation of hemiparesis	70	Not given	3 months (dead)
03	63/M	Left parietal and spenium of CC	AMS	60	NTR	None	80	External beam radiation-60Gy, Tab Temozolomide	26 months (alive)
04	68/M	Left frontal	AMS	80	GTR	None	90	External beam radiation-50Gy, Tab Temozolomide (RT not completed)	13 months (dead)
05	41/M	Left frontal	AMS, hemiparesis	60	GTR	None	80	External beam radiation-60Gy, Tab Temozolomide	20 months (alive)
06	51/M	Left parietal	Hemiparesis, speech difficulty	70	NTR	Transient aggravation of hemiparesis	90	External beam radiation-60Gy, Tab Temozolomide	18 months (alive)
07.	40/F	Right parietal	Hemiparesis	60	GTR	Transient aggravation of hemiparesis	80	External beam radiation-60Gy, Tab Temozolomide	20 months (dead)
08.	65/M	Right temporo-occipital	AMS, hemiparesis	60	PR	None	70	Not given	3 months (dead)
09	73/M	Right temporo-parietal	AMS, hemiparesis	50	PR	None	60	Not given	3 months (dead)
10	65/F	Left fronto-parietal	AMS, hemiparesis	60	GTR	Transient aggravation of hemiparesis	80	Not given	5 months (alive)
11	57/M	Left frontal	AMS	70	GTR	None	90	External beam radiation-60Gy, Tab Temozolomide	21 months (alive)
12	50/M	Right temporo-parietal	AMS	70	GTR	Tumor bed hematoma	80	External beam radiation-60Gy, Tab Temozolomide	6 months (alive)

Table 2

Demographic data and presenting characteristic of the patients.

Characteristics	Category	Frequency (%)/ Mean (range)
Age		57 (38–73)
Sex	Male	9 (75%)
	Female	3 (25%)
Tumor location	Right temporal	1 (8%)
	Right parieto-occipital	2 (17%)
	Right parietal	1 (8%)
	Right temporo-parietal	2 (17%)
	Left parietal and spenium of CC	1 (8%)
	Left frontal	3 (25%)
	Left parietal	1 (8%)
	Left fronto-parietal	1 (8%)
Presentation	AMS	4 (33%)
	AMS, hemiparesis	5 (42%)
	Hemiparesis	2 (17%)
	Hemiparesis, speech difficulty	1 (8%)
Preoperative KPS		62.5 (50–80)

3–26 months), during which patients were monitored for clinical progress and recurrence.

Surgical outcomes

The average duration of the surgical procedure was 180 min (range: 160–220 min), with a median hospital stay of 7 days (range: 5–10 days). Gross total resection (GTR) was achieved in 66.66% ($n=8$) of patients, near-total resection (NTR) in 16.66% ($n=2$), and partial resection (PR) in 16.66% ($n=2$) of cases (Table 3). The gross total resection (GTR) rate 66.7%, with a 95% CI ranging from 40.0 to 93.3%. This wide interval suggests variability likely due to the small sample size. Additionally, there was a statistically significant improvement in postoperative Karnofsky performance status (KPS) scores, with a mean increase of 16.7 points (95% CI: 13.9–19.5; P-value = 1.47×10^-7), indicating that patients generally experienced better functional outcomes after surgery. However, no statistically significant difference in overall survival was found between the GTR and non-GTR groups (P-value = 0.78), which may also be attributed to the limited sample size and the heterogeneity of the patient population. These findings underscore the importance of larger-scale studies to further elucidate the impact of resection extent on functional recovery and survival outcomes in this patient population.

Table 3

Summary of the results of glioblastoma surgery.

Characteristics	Category	Frequency (%)/ Mean (range)
Surgery duration		180 min (160–220)
Hospital stays		7 days (5–10)
Tumor resection	GTR	8 (67%)
	NTR	2 (17%)
	PR	2 (17%)
Complications	Transient aggravation of hemiparesis	4 (33%)
	Tumor bed hematoma	1 (8%)
	None	7 (58%)
Postoperative KPS		81.7 (60–100).

Adverse events

Despite these thorough monitoring protocols, the adverse effects observed in our cohort were minimal and transient. The most commonly reported side effect was a temporary yellow discoloration of the skin, sclera, and urine, which resolved within 24–36 h postadministration. One patient experienced a postoperative seizure, which was managed without long-term consequences. Importantly, no significant late-onset complications, such as delayed neurotoxicity, organ dysfunction, or persistent neurological deficits, were observed during the follow-up period. Routine blood and urine examinations revealed no abnormal changes, with normal liver and kidney function tests in all patients. These findings align with the existing literature on the safety profile of FS, which consistently reports a low incidence of adverse effects.

Clinical complications

Serious complications were reported in three patients: one with tumor bed hematoma, one with sepsis, and one with postoperative seizure. Four patients experienced transient aggravation of hemiparesis, which mostly resolved within one month. No perioperative mortality occurred during the study period.

Discussion

Since radical removal of a malignant tumor enhances long-term survival, the significance of radical resection in glioma surgery has already been highlighted in the literature[19,20]. Fluorescent markers have grown in importance as a surgical tool for neoplastic excision. This method is becoming more and more well-liked as a contemporary tool for achieving drastic elimination. For over 60 years, FS, a fluorophore, has been utilized in medical applications[21]. This dye allows for the real-time evaluation of the gadolinium-enhanced brain regions in MRI by penetrating the brain regions where the blood-brain barrier (BBB) is disrupted. It has been shown in an experimental investigation that intra-ar terial mannitol treatment disrupts the mouse BBB and increases the fluorescein signal in the brain[22].

According to prior research, fluorescein injection appears to be a useful technique for achieving a high GTR during surgery for malignant brain tumors, resection rate ranged from 75 to 100%[23]. Thirty-two patients receiving surgical treatment for glioblastoma multiforme were examined by Shinoda *et al.*[24] GTR was attained in 84.4% of these individuals with a high-dose of FS (20 mg/kg body weight). This result supports the large fraction of GTR that we have also attained. In order to assess the impact of intraoperative FS usage on the degree of GTR, overall prognosis, and side effects, Koc *et al.* reported a prospective nonrandomized trial. Following a craniotomy, 47 of the 80 patients who were recruited got a high dosage of intravenous FS (20 mg/kg body weight). The number of patients with GTR increased significantly (83 vs. 55%) when fluorescein was employed, according to the results[25].

When FS (15–20 mg/kg body weight) was used to treat patients, Chen *et al.* (2012) demonstrated a substantial improvement in progression-free survival and the GTR rate when compared to the control group. Our research likewise revealed a respectable survival rate[26]. Additionally, Okuda *et al.* reported on the efficacy and safety of a novel fluorescence-guided surgery method for GBM surgery based on high-dose FS using barrier filters and excitation on an operating microscope (OME9000

Olympus)[27]. Additionally, our research demonstrated the safety and effectiveness of FS in glioblastoma resection, permitting a high GTR rate. This suggests that FS makes it possible to identify tumor vessels and normal vessels and to conduct a thorough tumor assessment. The dye made it possible to carry out the sur gical removal under both standard white xenonlight illumination and fluorescence[27].

Kuroiwa *et al*.[28] was the first to report on a revolutionary method of fluorescence filter integration in a Zeiss microscope. A study by Schebesch *et al*. (2013) involved 35 patients with malignant brain tumors (22 HGGs) who had surgery and were given a low dose of FS (3–4 mg/kg) while utilizing a PENTERO 900 microscope fitted with a fluorescence light filter that had a wavelength of 560 nm. Tissue fluorescence was brilliantly apparent in all cases 30 min after FS was administered, and it persisted the entire process, suggesting a useful method for maximal tumor removal[16].

Acerbi *et al*. was the first group to initiate a prospective phase II trial (FLUOGLIO) in 20 consecutive patients with HGGs. With a 6-month progression-free survival rate of 71.4% and an 11-month median survival, 80% of patients underwent total tumor resection[23]. In a series provided by Hamamcıoğlu *et al*. 200 mg (2–4 mg/kg) of FS was used to surgically resect seven metastatic tumors and 23 high-grade tumors. For tumor delineation, FS was deemed 'helpful' in 29 of the 30 procedures (97%). A total resection (radiologically demonstrated) was achieved in 79% of patients regardless of the histopathology, whereas a near-total resection was achieved in four patients (14%)[29].

Several studies have established the efficacy of 5-ALA as a surgical adjunct, with GTR rates typically ranging from 65 to 85%. For instance, Stummer *et al*. (2006) reported a GTR rate of ~65% in a large cohort of glioblastoma patients treated with 5-ALA. In comparison, our study found a GTR rate of 66.6% with FS, which aligns closely with the outcomes observed with 5-ALA, suggesting that FS is a comparable alternative for achieving extensive tumor resection[5]. Moreover, Acerbi *et al*. (2013) investigated the use of FS in glioma surgeries and reported a 6-month progression-free survival (PFS) rate of 71.4% and an 11-month median overall survival. Our study's outcomes, with a median follow-up period of 13.4 months, also demonstrate favorable short-term survival rates, although we recognize the need for longer follow-up to fully assess long-term outcomes[23]. Chen *et al*. (2012) also demon strated that FS significantly improved GTR rates and PFS when compared to traditional white-light surgery, reinforcing the effectiveness of FS as observed in our study[26].

Additionally, the use of FS has been reported to be cost-effective and easier to administer compared to 5-ALA, which requires preoperative oral administration and is associated with higher costs and the risk of photosensitivity. Francaviglia *et al*. (2017) highlighted these advantages of FS, noting that it provides a reliable and safe alternative for glioblastoma resection, particularly in resource-limited settings where 5-ALA may not be readily available[10].

Our experience has shown that using FS in conjunction with a YELLOW 560 nm filter is 'helpful' for tumor removal throughout all surgical operations, since it improves the ability to distinguish between yellow-stained tumor tissue and pinkish brain tissue. Tumoral tissue staining was evident as soon as the dural opening was made, typically 30 min after the FS was administered. This staining persisted until the tumor was completely removed, and it matched the contrast enhancement of the preoperative MRI. Our study's tumor clearance rate was comparable to earlier research. Of the patients, 66.6% had GTR, 16.66% who had fluorescence-guided surgery obtained a STR (>95%). Moreover, FS seemed to be safe and effective in the intraoperative visual phase by distinguishing tumor from normal brain tissue and in the postoperative neuroimaging control. The resection was also maximized with the aid of neuro-navigation system in eloquent areas.

One FS administration is about 200 times less expensive than one 5ALA application[30]. Optimal delineation of tumoral tissue is made possible by the employment of particular filters on surgical microscopes. This ensures that fluorescent areas are visible and helps differentiate tumoral tissue from normal arteries and the peritumoral brain parenchyma, which appear to have 'more natural' colors[4]. It should be noted that BBB breakdowns, which correlate to the increased areas in MRI, are visible with fluores cence technology. Consequently, the neural tissue sections in which the BBB is disrupted can be removed using a resection based on FS fluorescence. It may decrease the precision of tumor identification since it does not always permit resection of the entire area of tumor cell infiltration. Furthermore, because the necrotic area of the tumor lacks capillaries, it does not stain with fluorescein[2,10].

Overall, compared to greater doses of FS or 5ALA, the low dose of FS combined with the YELLOW 560 nm filter provided for quicker intraoperative management without the need to wait for the dye peak[3,16]. With the exception of a predictable transitional shift in skin, sclera, and urine color that turned slightly yellow and vanished entirely 24 h after fluorescein was administered, none of the patients experienced any systemic or local adverse effects. Our results are encouraging, despite the small number of patients participating and the absence of a control group consisting of individuals undergoing surgery without fluorescein (which we acknowledge is not a secondary aspect). It is evident that the use of intravenous fluorescein with a particular yellow filter during the surgical excision of HGGs is a cost-effective, safe, and efficient method of achieving high GTR.

The absence of a control group is a significant limitation. However, several studies in the field of neurosurgery, including those by Shinoda *et al*. (2003), Acerbi *et al*. (2013), Schebesch *et al*. (2013), and Acerbi *et al*. (2018), have successfully provided valuable insights without a control group[23,24,31]. The inclusion of a control group in our study was challenging due to ethical concerns. Including a control group that does not receive FS might raise ethical concerns, particularly when FS has shown potential benefits in improving the extent of tumor resection, the surgical team has a preference for using FS. Denying some patients, the potential advantage of FS could be seen as withholding a beneficial intervention, which complicates the ethical approval process. Additionally, recruitment difficulties and logistical constraints made it impractical to include a control group in our single-center study. We compared our results with other studies using 5-ALA, a well-established surgical adjunct, and found that our GTR rate and short-term outcomes with FS were comparable. For instance, the GTR rates in our study were similar to those reported by Stummer *et al*. (2006) and Acerbi *et al*. (2013) using 5-ALA, which suggests that FS is an effective alternative. Acerbi *et al*. (2013) also added advantage of FS being more cost-effective and easier to administer, as it does not require oral intake hours before surgery[5,23,24,31].

In the context of glioblastoma research, many studies face

challenges in extending follow-up periods due to the aggressive nature of the disease, high recurrence rates, and patient attrition. For instance, studies such as those by Shinoda *et al.* (2003) and Okuda *et al.* (2012) reported median follow-up periods of around 12–15 months, similar to our study[25,27]. While these follow-up periods provide valuable insights into short-term and early postoperative outcomes, they are indeed limited in their ability to assess long-term survival and recurrence rates comprehensively. Similarly, studies like those by Stummer *et al.* (2006) with a median follow-up of 19.4 months and Acerbi *et al.* (2013) reporting 6-month PFS, have contributed to the understanding of surgical adjuncts like 5-ALA and FS, despite having follow-up periods that may not fully capture long-term outcomes[5,23]. Future research should aim to establish standardized monitoring protocols across institutions to ensure consistent and reliable detection of both immediate and late-onset complications asso ciated with FS.

Surgeries conducted by a single, highly experienced neurosurgeon ensures consistency in surgical technique but introduces a potential bias, as the outcomes observed may be partially attributed to the surgeon's expertise rather than solely to the use of FS. In our study, the tumor locations were diverse, including both superficial and eloquent areas. However, we did not stratify our analysis based on tumor location, which could have provided more nuanced insights into how FS performs in different anatomical contexts. We included patients with varying levels of comorbidities, the study did not perform a detailed subgroup analysis to determine the impact of these comorbidities on surgical outcomes. One of the key advantages of this research is its clinical relevance; FS provides a cost-effective and accessible alternative to 5-aminolevulinic acid (5-ALA). The study's detailed methodology, including standardized protocols for FS administration and rigorous monitoring of adverse effects, ensures the reliability of the findings and enhances the reproducibility of the research. By comparing the outcomes of FS with 5-ALA, this study contributes to the growing body of evidence supporting fluorescence-guided surgery, offering practical implications for improving surgical outcomes in glioblastoma patients.

Overall, FS offers a cost-effective and accessible alternative to 5-ALA, making it particularly valuable in settings where resources may be limited. However, while the initial results are encouraging, they highlight the need for further research to confirm the long-term efficacy and safety of FS in glioblastoma surgery. Future studies should focus on expanding the patient cohort, incorporating control groups, and extending follow-up periods to better assess recurrence rates and overall survival. Additionally, research should explore the potential benefits of FS in combination with other surgical adjuncts and investigate its use in diverse patient populations and tumor locations. Addressing these areas will help to establish FS as a standard of care in neurosurgical oncology and optimize surgical strategies for glioblastoma treatment.

Our study provides novel insights by demonstrating its safety, efficacy, and high resection rates in a broader patient cohort, including those with diverse comorbidities and varying tumor locations, which is less represented in previous research. Notably, our findings show that a standardized low-dose FS protocol (5 mg/kg) combined with the YELLOW 560 nm filter achieves a gross total resection (GTR) rate of 66.6%, comparable to outcomes reported with higher doses, suggesting that lower doses can effectively minimize potential side effects while maintaining efficacy. Additionally, our study highlights the cost-effectiveness and feasibility of FS in resource-limited settings, offers practical intraoperative protocol optimizations, and provides extended follow-up data on both short-term and long-term outcomes, thereby enhancing the understanding and applicability of FS-guided surgery in diverse clinical contexts.

Limitations

The small sample size of 12 patients restricts the generalizability of the findings and reduces the statistical power of the study. Additionally, the absence of a control group precludes direct comparisons with standard resection techniques, making it difficult to attribute the observed outcomes solely to the use of FS. The relatively short follow-up period of 13.4 months may also limit the assessment of long-term outcomes such as recurrence rates and overall survival. Furthermore, potential biases and confounding factors, such as surgeon experience and tumor location, were not fully controlled, which could influence the study's conclusions. Future research with larger cohorts, control groups, and extended follow-up periods is necessary to further validate the efficacy and safety of FS in glioblastoma surgery.

A larger cohort would be necessary to draw more robust and statistically significant conclusions. Future studies with increased sample sizes could help validate these findings and provide a broader understanding of FS's role in glioblastoma surgery. The absence of a control group in this quasi-experimental study is a significant limitation. Without a comparison group undergoing standard resection without FS, it is challenging to attribute the observed surgical outcomes solely to the use of FS. The inclusion of a control group in future research would allow for a more rigorous evaluation of FS's effectiveness and its impact on surgical outcomes.

Another limitation is the relatively short follow-up period of 13.4 months. Glioblastoma is known for its aggressive nature and high recurrence rates, and a longer follow-up period is necessary to fully assess the long-term safety and efficacy of FS-aided resection. Extended follow-up in future studies would provide better insights into the recurrence rates, progression-free survival, and overall survival associated with FS use. Moreover, the study's reliance on noncontrast CT scans for postoperative evaluation, rather than the gold standard of gadolinium-enhanced MRI, may have affected the accuracy of assessing residual tumor tissue. Although noncontrast CT was used for its accessibility and to reduce the risk of complications associated with gadolinium use, its limitations in detecting residual glioma are acknowledged. Future studies should consider using gadolinium-enhanced MRI to provide a more precise evaluation of the extent of tumor resection.

Finally, the study does not fully address potential confounding factors such as surgeon experience, tumor location, and patient comorbidities, which could influence the outcomes. While efforts were made to standardize the surgical procedure, these variables may still introduce bias. Future studies should include a more detailed analysis of patient health status and comorbidities, potentially adjusting for these variables in statistical analyses to reduce their confounding effect.

Conclusions

Our study demonstrates the safety, efficacy, and cost-effectiveness of fluorescein sodium (FS)-aided resection in the surgical

treatment of glioblastoma. Through a comprehensive analysis of 12 patients undergoing FS-guided surgery, we observed a high rate of gross total resection (GTR), with minimal adverse effects recorded. The integration of FS with specific fluorescence filters offers a practical and efficient approach for maximizing tumor removal while ensuring optimal tissue discrimination. Despite the limitations inherent in our study, including the absence of a control group, our findings align with existing literature, underscoring the utility of FS in enhancing surgical outcomes for glioblastoma patients.

Ethical approval

Ethical approval for this study (Ref: ICHRI/Research/ERC/2021/03) was provided by the Ethical Committee Ibrahim Cardiac Hospital and research hospital, Dhaka, Bangladesh on 21 July 2021.

Consent

Written informed consent was obtained from the patient for publication and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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All authors have contributed equally.

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The authors declare no conflicts of interest.

Research registration unique identifying number (UIN)

1. Name of the registry: not applicable.
2. Unique identifying number or registration ID: not applicable.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): not applicable.

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