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Journal of Neurosciences in Rural Practice

Original Article

Fluorescein-guided resection versus non-fluorescein-guided resection of high-grade gliomas: A prospective randomized controlled study

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ABSTRACT

Objectives: The objective is to quantify the extent of resection (EOR) and assess the survival of High-grade gliomas (HGG) using intraoperative fluorescein sodium compared to non-fluoresceinguided resection using pre-operative and post-operative contrast-enhanced magnetic resonance imaging.

Materials and Methods: A prospective randomized controlled study was carried out in which the study group underwent florescence guided surgery (FGS) and was compared with non-FGS control group. Evaluation was done on EOR and gross total resection (GTR) based on radiological imaging. Karnofsky performance status (KPS), length of hospital stay, and survival rate were also evaluated.

Results: Thirty-two patients (16 in each group) with Grade 3/4 gliomas with comparable pre-operative clinical features and tumor volume were recruited. In the study group, the mean EOR was found to be 95.13% and the control group showed an EOR of 85.19%, which was statistically significant ($P = 0.046$). There was also a statistically significant difference in GTR rates ($P = 0.046$) and KPS at 3 months (0.050) between the two groups.

Conclusion: Microscopic fluorescein-guided resection of HGGs is feasible and safe. The present study was able to demonstrate higher rates of EOR and GTR in the FGS group, which resulted in a higher KPS score as compared to the non-FGS group.

Keywords: Extent of resection, Fluorescein, Gross total resection, High-grade glioma

INTRODUCTION

There is a growing incidence of central nervous system (CNS) tumors, which range from 5 to 10/100,000 people and represent 2% of all malignancies.[1] High-grade gliomas (HGGs) represent 14.5% of all CNS tumors and 48.6% of malignant CNS tumors, making them one of the most aggressive cancers and the most frequent malignant primary tumor of the brain and CNS.[2] Resection rate has been found to be significantly correlated with overall survival (OS) without progression, neurological impairment, or seizures. Furthermore, complete excision of the tumor increases the efficacy of adjuvant treatments.[3,4] Gross total resection (GTR) (extent of resection – extent of resection (EOR) = 100%) was associated with a 2–8-month increased survival benefit compared to subtotal resections (EOR = 50–98%) according to the literature.[3-5] However, complete surgical resection becomes a difficult task due to the aggressive nature of these tumors, which infiltrate the surrounding normal brain tissue.[3-7]

To optimize the EOR, neurosurgeons have adopted a number of intraoperative techniques such as the administration of 5-Aminolevulinic acid (5-ALA) and the use of intraoperative magnetic resonance imaging to guide resection.[8,9] Both these modalities have significant costs associated with them and are not feasible in a resource-constrained setting. A cost-effective modality described is surgery guided by fluorescence.[10-12] Fluorescein penetrates the high-grade tumor cells due to the disrupted blood–brain barrier (BBB). Tumor is then visualized using a YELLOW 560 nm surgical microscope filter. Fluorescence-guided surgery (FGS) provides real-time visualization of tumor margins, aiding neurosurgeons in distinguishing between malignant tissue and surrounding healthy brain parenchyma. The potential benefits of this technique include improved precision in tumor removal, thereby enhancing patient outcomes. Several studies have described the usefulness of this technique in enhancing the EOR of HGGs.[10-12]

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Received: 23 May 2025 **Accepted:** 02 November 2025 **Epub ahead of print:** 07 February 2026 **Published:** 12 March 2026 **DOI:** 10.25259/JNRP_196_2025

However, there is no prospective randomized controlled study quantifying fluorescein sodium-guided resection compared to non-fluorescein sodium-guided resection of high-grade supratentorial gliomas.[10-12] This study aimed to assess and compare the extent of tumor resection, intraoperative complications, post-operative neurological outcomes, and OS between patients undergoing fluorescein-guided resection and those undergoing non-fluorescein-guided resection for HGGs.

MATERIALS AND METHODS

Study setting

Patients operated between January 2022 and June 2023, in the Department of Neurosurgery/Emergency in a tertiary care center, and fulfilled the inclusion criteria, were enrolled in the study after written informed consent. Ethical clearance was obtained from the institutional board.

Study design

Prospective randomized controlled study.

Study group

Patients with a supratentorial HGG in whom intraoperative fluorescein sodium was used for resection.

Control group

Patients with a supratentorial HGG in whom fluorescein sodium was not used intraoperatively.

Inclusion criteria

- Patients of both genders, and above the age of 18 years.
- Patients with suspected high-grade untreated single lesions of the supratentorial region of the brain, as suggested by pre-operative magnetic resonance imaging with intravenous contrast agent administration.
- Patients who gave written informed consent for the study.

Exclusion criteria

- Previously operated lesions
- Multicentric tumors
- Presence of active malignant tumors at any other site
- All tumors that turned out to have a biopsy other than HGGs on histopathological diagnosis after resection (HGG refers to tumors that are classified as World Health Organization Grade III and Grade IV)
- Known allergy to contrast agents or history of previous anaphylactic shocks
- Known severe previous adverse reactions to fluorescein sodium
- Women in their first trimester of pregnancy or lactation
- Patients with deranged kidney or liver function tests.

Methodology

The patients fulfilling the inclusion criteria were randomized into two groups. For randomization, computer-generated randomization tables were used, in which each set consisted of 16 unique numbers each. The demographic features and pre-operative clinical findings were noted. All patients underwent a pre-operative MRI study (with volumetric sequences) with and without the use of a contrast agent. After intubation and before skin incision, patients allotted to the study group received 5 mg/kg of a 20% solution of sodium fluorescein administered intravenously. All patients received pre-operative steroids in both groups. There was no anaphylactic reaction or adverse event due to FS administered to the patients. A dedicated surgical microscope (Pentero 800, Zeiss Kinevo 900, and Leica OHX with fluorescence kit, YELLOW 560) was used during the surgical procedure. Neuronavigation (if used) was used only for surgical planning, initial tumor localization, and orientation during tumor removal, but not for judgment regarding EOR. Intraoperative findings (duration of surgery, blood loss) post-operative complications, and length of hospital stay were noted. The post-operative MRI studies were done within the first 72 h after surgery [Figures 1 and 2]. The pre-operative tumor volume and the post-operative residual tumor volume were calculated by two independent neuroradiologists blinded to the surgical group (T1 IR, T1 Post-contrast Bravo, and 3D fluid attenuated inversion recovery (FLAIR) sequences), and the percentage of residual volume was compared in both groups. GTR was considered the absence of contrast enhancement on post-operative imaging, and EOR was calculated accordingly based on the contrast enhancement noted on post-operative scan. The 2/3 SH rule was utilized for measurement of volume, where S stands for maximally involved slice and H stands for height in a contrast-enhanced T1-weighted sequence using the DICOM software. Additionally, the status of the patient at 3 months follow-up was evaluated using the KPS score by an assessor independent of the operating team. The OS of the patients at the last known follow-up was noted.

Statistical analysis

Data collected during the study was compiled using Microsoft Excel spreadsheets. Data was entered and analyzed using Statistical Package for the Social Sciences (SPSS) International Business Machines Corporation (IBM) software version 23 (IBM SPSS Advanced Statistics, Chicago, IL, USA). Normality of data was tested with the Shapiro–Wilk test. Continuous variables: Student's t-test or Mann–Whitney U-test. Categorical variables: χ^2 or Fisher's

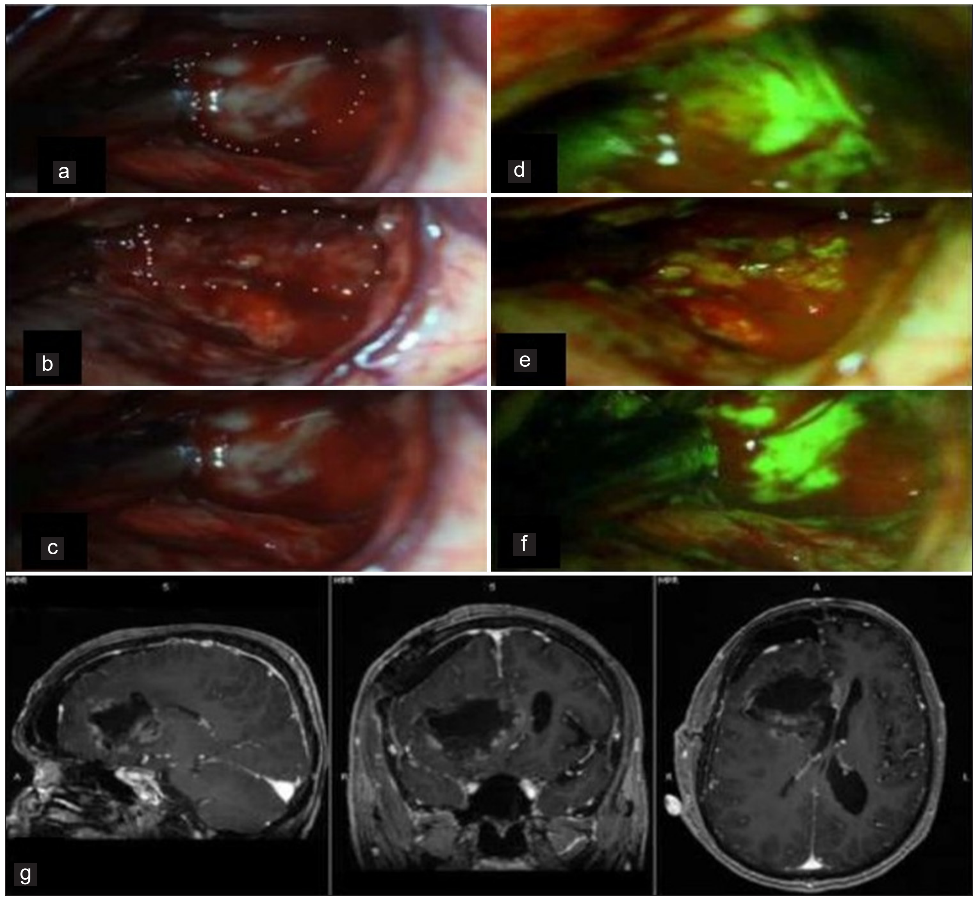

Figure 1: (a-c) Tumor tissue visualized under white light microscopy (marked with white dotted line). (d-f) Corresponding tumor tissue seen under fluorescein filter; (g) Post operative contrast-enhanced magnetic resonance imaging showing gross total resection.

exact test. For Survival, Kaplan–Meier curves with log-rank test, Cox proportional hazards models adjusted for tumor grade (III vs. IV) and adjuvant therapy compliance. A *post hoc* power analysis (using observed hazard ratios for OS) estimated power at approximately 58% to detect an Hazard Ratio (HR) = 0.6 at $\alpha = 0.05$, confirming that the study was statistically underpowered. p value < 0.05 was considered statistically significant.

RESULTS

Thirty-two patients (16 per group) met the inclusion criteria. Baseline demographics and clinical features were comparable [Tables 1 and 2]. The CONSORT diagram for the study participants is shown in Figure 3. Tumor locations were predominantly frontal (9 vs. 7), followed by parietal (3 vs. 2) and temporal (2 vs. 3). Eloquent cortex involvement did not differ significantly (3 vs. 4).

The mean duration of surgery [Table 3] and blood loss were comparable. No fluorescein-related adverse events were observed.

Histopathology revealed Grade III tumors in 2 (12.5%) of the study group versus 5 (31.3%) of controls, and Grade IV in 14 (87.5%) versus 11 (68.8%), respectively — a distribution that may confound survival outcomes.

The mean EOR was 95.1 ± 4.8% in the fluorescein group versus 85.2 ± 9.2% in controls ($p = 0.046$) [Table 4]. GTR was achieved in 8 (50%) versus 4 (25%) patients ($p = 0.046$). KPS at 3 months was significantly higher in the fluorescein group. Adjuvant therapy compliance differed: 2 (12.5%) study versus 6 (37.5%) control patients defaulted therapy due to socioeconomic factors and limited access to radiotherapy. After adjusting for adjuvant therapy status, survival differences remained non-significant.

Mean OS was 10.4 months (study) versus 8.1 months (control), with no statistically significant difference (log-rank $p = 0.26$), though numerically higher survival was noted in the fluorescein group [Figure 4].

DISCUSSION

Due to technological advancements in neuroradiology and dye materials, the use of fluorescent markers during surgical

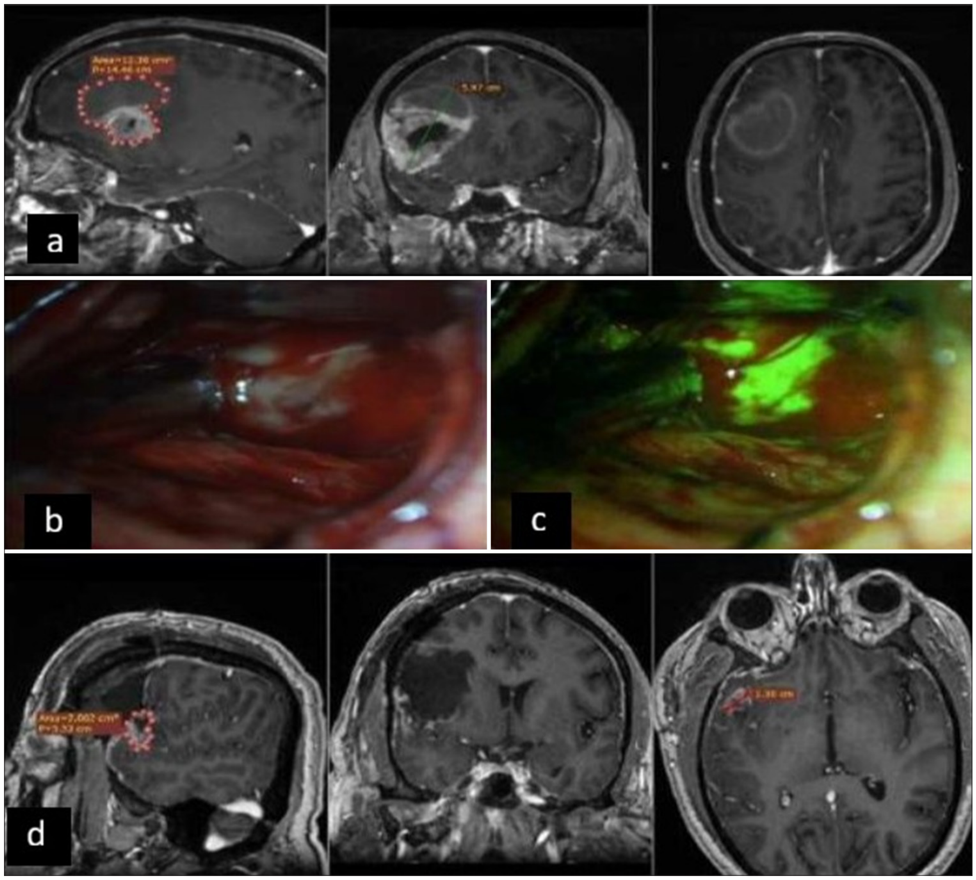

Figure 2: (a) Pre-operative contrast-enhanced magnetic resonance imaging (CEMRI) showing the right frontal lesion with a dotted line; (b) white light microscopic view of the lesion; (c) Corresponding tumor tissue seen under fluorescein filter, (d) Post operative CEMRI suggestive of residual lesion with a dotted line.

Table 1: Demography data.

Name of variables	Mean±S.D. (years)		*p*-value
	Study group	Control group	
Age (years)	48.75±13.09	51.06±14.86	0.644
Gender	*n* (%)		0.694
Male	11 (68.7)	12 (75)	
Female	5 (31.3)	4 (25)	

SD: Standard deviation, *p*-value significant threshold is <0.05

procedures to dye tumor tissue has become an important tool in neoplastic resection.[10-12] 5-ALA has been approved by the U.S. Food and Drug Administration for intraoperative use. However, the high cost, along with the risk of photosensitivity, has restricted its use, especially in the resource-constrained setting.[10-12]

Fluorescein is a low-cost and safe alternative for tumor delineation of HGGs. It is able to penetrate and accumulate in tumor cells due to the disrupted BBB and has been found to be a surrogate marker for Gadolinium enhancement in T1W MRI of HGGs.[10-12] Our randomized controlled study was able to demonstrate a significantly higher GTR rate (50%) and EOR (95.13%) in patients operated using the fluorescein-guided technique as compared to the control group (GTR rate – 25%, EOR – 85.19%). Earlier studies on the topic utilized high-dose fluorescein at a rate of 20 mg/kg, which makes it visible to the naked eye without the use of special microscopic filters. Koc *et al.* conducted a prospective non-randomized study with 47 patients in the fluorescein group and 33 in the non-fluorescein group.[13] GTR rates were 83% and 55%, respectively. Similar significant difference in GTR rates as well as progression-free survival was reported by Chen *et al.*, albeit in a relatively small cohort of 22 patients.[14]

Studies conducted in the last decade have utilized low-dose fluorescein (5 mg/kg) with a dedicated microscope filter. A multicenter prospective study Fluorescein Guided Surgery for Resection of High Grade Gliomas: A Multicentric Prospective Phase 2 Study (FLUOGLIO) without a control group recruited 57 patients with a mean tumor volume of 28.75 cm^3.[15] Thirty-eight patients (82.6%) underwent GTR. This study demonstrated FGS to be a safe

Table 2: Pre-operative clinical features.

S. No.	Name of variables	Frequency in total	Study group (%)	Control group (%)	*p*-value
1	Headache	24	14 (87.5)	10 (62.5)	0.102
2	Vomiting	22	13 (81.3)	9 (56.3)	0.127
3	Diminution of vision	6	5 (31.3)	1 (6.3)	0.070
4	Diplopia	4	3 (18.7)	1 (6.3)	0.285
5	Seizure	15	10 (62.5)	5 (31.3)	0.077
6	Memory disturbance	13	6 (37.5)	7 (43.7)	0.719
7	Motor deficit	17	6 (37.5)	11 (68.7)	0.077
8	Sensory deficit	4	2 (12.5)	2 (12.5)	--
9	Urinary incontinence	5	2 (12.5)	3 (18.7)	0.626
10	Co-morbidities	8			0.640
	CAD	1	0	1 (6.3%)	
	DM	2	1 (6.3)	1 (6.3)	
	Hypertension	2	1 (6.3)	1 (6.3)	
	Smoking and opium use	3	0	3 (18.7)	
11	Lobar signs	11	6 (37.5)	5 (31.3)	0.710
12	Papilledema	9	5 (31.3)	4 (25)	0.694
13	Antiepileptic drugs used	15	10 (62.5)	5 (31.3)	0.077
14	Midline shift in preoperative imaging	27	15 (93.7)	12 (75)	0.144

CAD: Coronary artery disease, DM: Diabetes mellitus, *p*-value significant threshold is <0.05

Table 3: Intraoperative findings in both groups.

Name of variables	Mean±S.D.		*p*-value
	Study group	Control group	
Duration of surgery (hours)	5.094±0.86	4.813±0.91	0.376
Blood loss (milliliter)	550.00±136.62	593.75±25.00	0.217
Length of hospital stay (days)	7±2.7	8±3.5	0.324
Pathological Grade of Tumor (%)			
Grade III	2 (12.5)	5 (31.25)	0.200
Grade IV	14 (87.5)	11 (68.75)	

p-value significant threshold is <0.05, SD: Standard deviation

and effective technique for the removal of HGGs. They also called for a randomized controlled trial to definitely confirm their findings. Katsevman *et al*. retrospectively compared their cohort of 57 patients who under FGS (from 2014 to 2017), to 132 patients who had undergone surgery without fluorescein (from 1999 to 2017).[16] The fluorescein guided surgery (FGS) group had significantly more rates of GTR and Near total resection (NTR) (73% vs. 53%) as well as median survival (78 weeks vs. 60 weeks), although comparison between two groups of patients operated so far apart (almost 20 years) has been criticized.[17] Schebesch *et al*. (2022) retrospectively analyzed patients

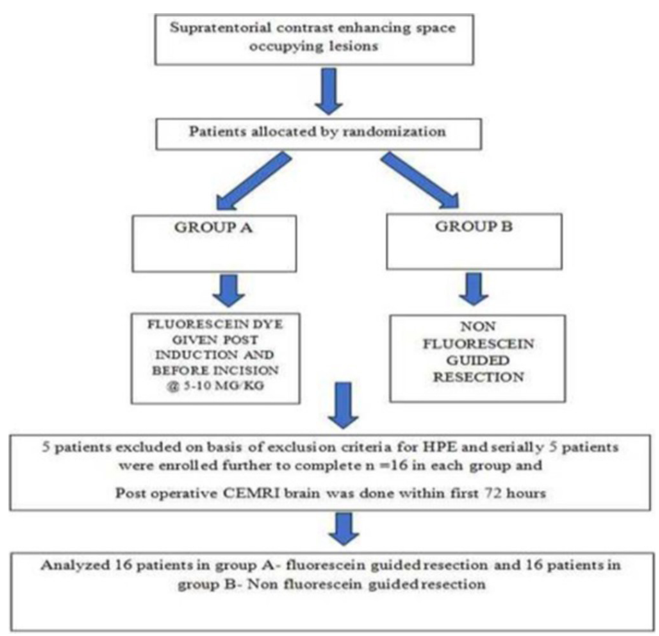

Figure 3: Consort diagram of study. CEMR: Contrast-Enhanced Magnetic Resonance Imaging, HPE: Histopathology examination

from an HGG registry.[17] They identified 196 patients who underwent FGS versus 151 patients who underwent white light microscopic resection. They found a significant difference in EOR as well as PFS between the two groups.

Table 4: Comparison of tumor volume, extent of resection, adjuvant therapy and survival.

Name of variables	Mean±SD		*p*-value
	Study group	Control group	
Pre-operative tumor volume (in cc)	54.50±27.85	42.98±29.48	0.265
Post-operative tumor Volume (in cc)	1.650±2.348	7.671±11.027	**0.041**
EOR (in %)	95.13±5.13	85.19±1.34	**0.046**
GTR	8 (50%)	4 (25%)	**0.046**
KPS at discharge	73±16	70±13	0.486
KPS at 3 months	72±20.424	56±20.586	**0.050**
Postoperative chemo and radiotherapy	14	10	-
No treatment postoperatively	2	6	-
Survival (in months)	10.4±5.3	8.2±5.1	0.212

SD: Standard deviation, EOR: Extent of resection, GTR: Gross total resection, KPS: Karnofsky performance status, Bold values indicate *p*-value clinically significant (<0.05), *p*- value significant threshold is <0.05

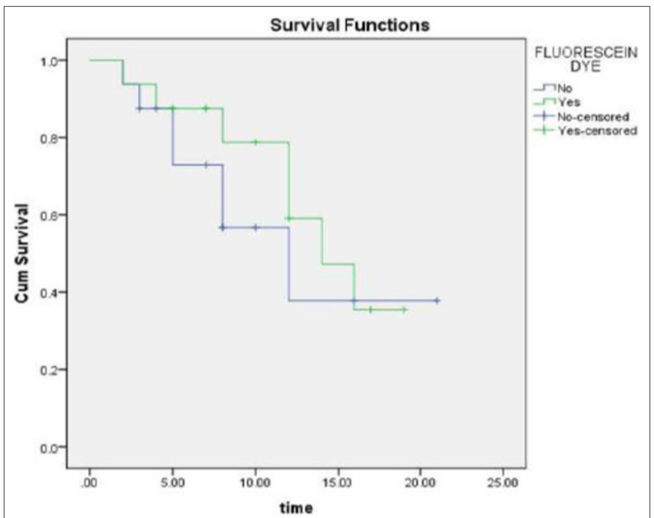

Figure 4: Kaplan meier curve depicting overall survival on follow up.

A similar retrospective analysis with 61 in the fluorescein group and 51 in the non-fluorescein group was reported by Xi *et al.* in 2023.[18] No significant difference in blood loss was seen between the two groups, as seen in our study, although a significantly shorter duration of surgery was reported in the fluorescein group. The GTR rate was similar to what was seen in our study (45.9% in FGS vs. 19.6% in non-FGS group). Another retrospective study reported significantly lower hemorrhage rates and operative times in the FGS group as compared to the control group. The rate of tumor recurrence at 6-month follow-up was also significantly lower in the FGS group (11.9% vs. 25.0%, p = 0.01).[19]

The absence of Class I evidence in the form of randomized controlled trials has led to many recent meta-analyses on the subject.[10-12] A cohort of 336 patients from 21 studies was analyzed, revealing a GTR rate of 81%. 10 case–control studies showed a 29.5% improvement in GTR rates with FGS.[11] A three-way comparison was performed between FGS, 5-ALA, and intraoperative MRI. The highest rate of GTR was seen with iMRI, while comparable rates were seen between FGS and 5-ALA.[12] Similar improvement was also seen in OS and PFS with both FGS and 5-ALA.

This study also highlights the unique constraints of management of HGG in the developing world. The immunohistochemistry of the tumor tissue could not be ascertained due to the unavailability of the kits in the pathology department. This makes maximizing the EOR all the more important in our cohort of patients, for which FGS is a relatively low cost option. Six patients in the control group and two in the study group could not receive adjuvant chemo-radiotherapy due to the socio-economic constraints and the non-availability of adequate oncology services in the region, thus potentially affecting the OS of patients.[20]

While the OS improvement did not reach statistical significance, the trend toward better outcomes aligns with previous evidence, suggesting that greater cytoreduction confers clinical benefit even when underpowered to show a statistical difference. In our study, the mean survival in patients of Fluorescein guided resection (10.43 months) was better than compared to non-fluorescein-guided resection (8.12 months), but statistically it was not found to be significant. This is similar to the findings of previous studies by Koc *et al.*[13]. On the other hand, a recent study by Schebesch *et al.*[17] was able to demonstrate significantly longer OS and PFS in the FGS group. This could be due to the large sample size analyzed (n = 347). Importantly, the study faced limitations related to small sample size, tumor grade imbalance, and variation in adjuvant therapy compliance, each of which could confound survival outcomes. The post-hoc power analysis confirmed that the study had <60% power to detect moderate survival differences, emphasizing the need for larger multicentric validation.

Although blinding was implemented for radiological and KPS assessments, complete blinding during surgery was not possible. This may have introduced minor observer bias.

Furthermore, the lack of molecular marker data isocitrate dehydrogenase (IDH mutation, 1p/19q co-deletion, Methylguanine DNA methyltransferase (MGMT) promoter methylation) limits the accuracy of prognostic interpretation, as these markers strongly influence survival and treatment response.

Nevertheless, within the context of a developing healthcare system, FGS represents a cost-effective, safe, and practical tool to enhance resection completeness and short-term functional outcomes.

Limitations of the study

- Small cohort ($n = 32$) limits statistical power and external validity
- Tumor grade imbalance (Grade III/IV) and adjuvant therapy non-compliance may confound survival outcomes
- Absence of molecular profiling restricts precision in prognostic analysis
- Incomplete blinding may have introduced assessment bias
- Despite these limitations, the observed survival trends have clinical relevance and merit exploration in larger, stratified studies.

CONCLUSION

Fluorescein-guided microsurgical resection of supratentorial HGGs significantly improved EOR and GTR rates and yielded better short-term functional outcomes compared with conventional resection. Although survival improvement did not achieve statistical significance due to limited power and treatment imbalance, the findings support FGS as a safe, affordable adjunct for glioma surgery in resource-limited settings.

Acknowledgment: We would like to acknowledge Mr. Nitin and Mr. Lal Singh, Operation Theater Technicians from Department of Neurosurgery for their technical assistance in the operation theater.

Ethical approval: The research/study was approved by the Institutional Review Board at All India Institute of Medical Sciences, approval number AIIMS/IEC/2022/5114, dated 26th August 2022.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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How to cite this article: Mistry J, Jain KS, Mourya AK, Agrawal M, Tiwari S, Jha D, *et al*. Fluorescein-guided resection versus non-fluorescein-guided resection of high-grade gliomas: A prospective randomized controlled study. J Neurosci Rural Pract. 2026;17:73-80. doi: 10.25259/JNRP_196_2025