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Clinical Impact of Sodium Fluorescein-Assisted Tumor Resection Using a 560-nm Microscope Filter in Patients with Glioma

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Abstract

Background: Sodium Fluorescence-guided resection remains controversial; therefore, the study aimed to evaluate its utility in various malignant gliomas using the YELLOW 560 nm Surgical Microscope Filter.

Methods: The study was conducted prospectively at the neurosurgical unit of a tertiary-care teaching institute in eastern India. The study consistently included adult patients of either sex with a presumed diagnosis of "primary" or "secondary" brain tumor on MRI without any co-morbidities, Karnofsky index higher than 80%, and no contraindications to contrast agent. Sodium fluorescein was used during surgical planning to guide tumor removal, with a fluorescent YELLOW 560-nm filter (Kinevo 900®, Carl Zeiss Meditec AG, Germany). Histological analysis of the tumors was performed, and postoperative MR images were ideally obtained within 48 hours of surgery to categorize resection under successful and unsuccessful groups due to residual lesions. Descriptive statistics were used to analyze the data.

Results: The study included 51 patients with a mean age of 52.6 years; one-fourth were elderly, and the remainder were adults. The median preoperative tumor volume was 16.6 cm3. Of the 51 patients, 44 (86.3%) had successful intraoperative tumor fluorescence. Gross total resection was achieved in 32 (62.7%) patients, followed by near total resection in 12 (23.5%) patients. Only 7 (13.7%) patients underwent subtotal resection. All patients histopathologically presenting LGG, oligodendroglioma, and choroid plexus papilloma tumors had residual lesions, making them unsuccessful after sodium fluorescein-guided tumor resection. The relative risk of unsuccessful resection was not seen in gender or tumor position. Sodium fluorescein-guided tumor pattern visualization showed bright, patchy, and homogeneous views in all enrolled patients, except in low-grade glioma (LGG), oligodendroglioma, and choroid plexus papilloma.

Conclusion: A modest dose of sodium fluorescein was successful in detecting and achieving gross and near-total resection of glioblastoma, high-grade gliomas, and metastatic tumors.

Keywords: Sodium Fluorescein; Malignant Gliomas; Fluorescence-Guided Surgery; Extent of Resection.

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Introduction

In India, the prevalence of central nervous system (CNS) tumors is from 5 to 10 per 100,000 people and is on the rise. CNS tumors account for approximately 2% of all malignancies. [1,2] In adult patients, gliomas are the most prevalent primary cerebral tumors. Gliomas occurred with an age-standardized incidence rate of 4.7 per 100,000 person-years.[3] The maximum resection done safely in case of gliomas is considered the gold standard of care.[4] Enhancing cytoreduction and minimizing neurologic deficits can be crucial when gliomas affect or approach expressive areas of the brain.[5] The cornerstone of treatment in the neurosurgical management of gliomas is maximal safe resection, and maintaining functional integrity is associated with successful outcomes. The effectiveness of the extent of resection (EOR), survival, and complications across various tumor types and grades remains unknown, despite advances in adjuvant modalities and technologies that have improved identification and visualization of the tumor-brain margin to support neurosurgeons in the operating room.[6]

One visual marker of tumors is Sodium Fluorescein, injected intravenously, which crosses areas of disrupted blood-brain barrier (BBB) to stain tumoral cells. In 1948, sodium fluorescein (SF) was first used to distinguish between several types of brain tumors.[7] Low dosages of sodium fluorescein can be used to visualize tumour tissue under the surgical microscope with the use of specific light filters, or high doses (20 mg per kilogram of body weight) can be used to visualize tissue under standard white microscopic illumination.[8] The clinical use of SF dye for glioma surgery is still undergoing clinical research and is not currently an FDA-approved application. The use of SF and other fluorescent markers has been reported in the literature, particularly in

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articles highlighting the surgical treatment of glioblastoma multiforme and brain metastatic disease.[9-11] In a small sample of these skull base lesions, SF as an adjuvant for surgical excision in 2010 was used.[12] Overall, while fluorescein has demonstrated potential in brain tumor surgery, further research and clinical studies are needed to determine its optimal use, specificity, and clinical benefits in guiding surgical resection.[13] The pursuit of novel procedures in neurosurgery is motivated by the need for better surgical results and the difficulties in accomplishing maximal safe resection in gliomas. By assessing the efficacy of intraoperative fluorescence-guided resection procedures using SF across a range of glioma types, this study seeks to further this exploration. By doing so, we aim to elucidate the potential advantages and challenges of integrating fluorescence guidance into glioma surgery, as well as its role in improving surgical outcomes. We also attempted to demonstrate the visualization across the various histopathological subtypes of gliomas.

Materials and Methods

In this non-randomized prospective observational study, after the approval of the Institutional Ethics Committee (ECR/35/Inst/WB/2013/RR-19-IEC 214) with ethical committee code IPGME&R/IEC/2022/214, with careful consideration of the particular goals and statistical techniques used in this investigation, the sample size of 51 patients was established. Factors such as the expected effect size, statistical power, and the significance level (α) were considered in the computation. All patients were surgically treated at the neurosurgical unit of Bangur Institute of Neurosciences, IPGMER & SSKM Hospital, Kolkata, West Bengal, India, over 18 months from May 2021 to November 2022. Each patient was informed of the study's benefits and risks and signed an informed consent form. The inclusion criteria were as follows: age 18 and older, the presumed diagnosis is “primary” or “secondary” brain tumor on MRI, regardless of WHO grading without any co-morbidities, Karnofsky index higher than 80%, no contraindications to contrast agent (severe liver and kidney diseases or an increase in the concentration of liver enzymes by 2 times or more, pregnancy, lactation). Patients with tumors originating from the midline and brainstem, patients diagnosed with metastatic cancer and multifocal lesions, and pregnant and lactating females were excluded.

Before surgery, the neurosurgeons assessed the feasibility of achieving Gross Total Resection (GTR) using conventional techniques, based on the tumor's location and proximity to eloquent structures. The surgeries were performed by experienced neurosurgeons who started incorporating sodium fluorescein for intracranial tumor surgeries once it became available at the institution.

Operative planning employed standard contrast-enhanced MRI sequences and neuronavigation. Sodium fluorescein, administered intravenously at 3-4 mg/kg, was used during surgery. The fluorescein-stained tissue was visualized using a 560-nm fluorescent YELLOW filter (Kinevo 900®, Carl Zeiss Meditec AG, Germany) (Figure 1). During the surgery, all areas exhibiting fluorescence (Figure 2) and considered safe for resection were removed. It was observed that areas of fluorescence, which appeared normal under white light microscopy, were frequently present, particularly at the tumor margins. Histological analysis of the tumors was performed in accordance with standard protocols [14] by an independent neuropathologist.

Postoperatively, patients were admitted to the Intensive Care Unit (ICU) for monitoring. Postoperative MR images were ideally obtained within 48 hours of surgery. Residual tumor was identified as hyperintensity on T1-weighted postcontrast imaging without corresponding hyperintensity on T1-weighted non-contrast sequences. The researchers categorized the degree of resection into three categories: Gross Total Resection (GTR) when no residual tumor was observed, Near-Total Resection (NTR) when greater than or



Figure 1. Kinevo 900®, Carl Zeiss Meditec AG, Germany.

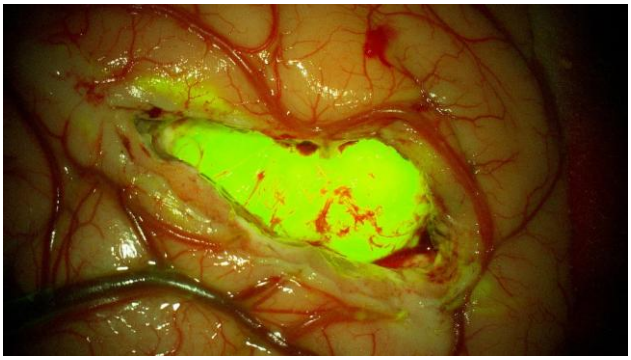


Figure 2. Tumor enhancing after giving Sodium Fluorescein dye under a yellow filter.

equal to 98% of the tumor was resected, and Subtotal Resection (STR) when less than 98% of the tumor was resected.[15] The tumor and residual volume were calculated preoperatively and postoperatively. The patients with a residual volume of tumor were considered to be unsuccessful after MRI detection within 1 month.

The patients were treated postoperatively in accordance with the Stupp protocol, which included postoperative radiosurgery, chemotherapy, radiation, and concurrent temozolomide therapy.[16]

Statistical Analysis

Microsoft Excel® 2010 was used for descriptive and analytical purposes. Mean, median, and standard deviation were used to analyze quantitative data. Percentages were used to describe qualitative data, and the Chi-square test was used to examine differences in proportions. Unpaired ' "t" tests were used to compare mean differences. Statistics were deemed significant at P 0.05. Epi Info™ was used for statistical analysis.

Results

This prospective study included 51 adult patients who underwent tumor resection guided by sodium fluorescein. The study included a diverse group of patients aged 19 to 77 years, with a female predominance of 33 (64.7%). The average (SD) age of the study subjects was 52.6 (13) years. One fourth (27.5%) of the patients were elderly, and the rest (72.5%) were adults.

Tumor data are summarized in Table1. The median (Q_1-Q_3) preoperative tumor volume was 16.6 (9.32-33) cm^3. At neuropathological analysis, 19 (37.3%) patients were diagnosed with glioblastoma (GBM), 5 (9.8%) patients with diffuse astrocytoma, 4 (7.8%) patients with low-grade glioma, 3 (5.8%) patients with hemangioblastoma, followed by 2 (3.9%) patients each with high-grade glioma and meningioma. The metastatic tumor was histopathologically characterized in 6 (11.8%) patients. The patients were also diagnosed with DNET, oligodendroglioma, choroid plexus papilloma, and ependymomas. The tumors were located in different areas of the patients' brains. Predominantly, the patients had tumors in the frontal region (13; 25.5%), the temporal region (11; 21.6%), the cerebellar region (5; 9.8%), and the insular, parietal, and temporoparietal regions (4; 7.8% each). A few other patients had tumors located at the insular, parietal, cerebellar, and corpus callosum regions as well. Tumors were also observed predominantly on the left side of the brain in 30 (58.8%) patients compared to the right 15 (29.4%). Of 51 patients (100%), 44 (86.3%) had successful intraoperative tumor fluorescence. Gross total resection was achieved in 32 (62.7%) patients, followed by near resection in 12 (23.5%). Only 7 (13.7%) patients had subtotal resection.

Distribution of patient characteristics by successful vs. unsuccessful outcomes with the fluorescence technique (based on residual or no residual after 1 month) is shown in Table 2. Among the 44 (86.3%) successful groups, 29 (65%) underwent GTR, with 1/4 achieving NTR. However, in the other group, 4 (57.1%) patients had NTR, and 3 (42.9%) had subtotal resection. All the patients' histopathologically presenting LGG, oligodendroglioma, and choroid plexus papilloma tumors had residual lesions and were hence unsuccessful after sodium fluorescein-guided tumor resection. The relative risk of unsuccessful resection was not associated with gender or tumor position. Among the 44 (86.3%) successful groups, 29 (65%) underwent GTR, with 1/4 achieving NTR. However, in the other group, 4 (57.1%) patients had NTR, and 3 (42.9%) had subtotal resection. A significant difference was observed (P = 0.003) between the successful and unsuccessful groups regarding gross and near-total resection.

Table 3 shows the Sodium Fluorescein tumor pattern

Table 1. Tumor data of study patients (N=51).

Characteristics	n (%)
Volume of tumor (in cm^3), median (Q_1-Q_3)	15.6 (13-22.5)
Histopathology	
Gliobastoma (GBM)	19 (37.3)
Hemangioblastoma	3 (5.8)
Low grade glioma (LGG)	4 (7.8)
High grade glioma (HGG)	2 (3.9)
Metastasis	6 (11.8)
Diffuse astrocytoma (DA)	5 (9.8)
Meningioma	2 (3.9)
Others	10 (19.6)
Location of Tumor	
Frontal	13 (25.5)
Temporal	11 (21.6)
Insular	4 (7.8)
Parietal	4 (7.8)
Cerebellar	5 (9.8)
Corpus Callosum	3 (5.8)
Temperoparietal	4 (7.8)
Others	7 (13.7)
Position/Laterality	
Left	30 (58.8)
Right	15 (29.4)
Bilateral	1 (1.9)
Degree of Resection	
Gross total resection (GTR)	32 (62.7)
Near total resection (NTR)	12 (23.5)
Subtotal resection	7 (13.7)

Table 2. Distribution of patients (N=51) characteristics with Successful vs. Unsuccessful by fluorescence technique (on the basis of residual or no residual after 1 month).

Characteristics	Successful n (%)	Unsuccessful n (%)	*P* value
Patients	44 (86.3)	7 (13.7)	
Age in years (mean, SD)	52.7 (13.2)	51.9 (14.1)	0.88
Male	14 (31.8)	4 (57.1)	0.22
Female	30 (68.2)	3 (42.9)	
Volume of tumor in cm^3, median (Q_1-Q_3)	16.75 (9.8-32.8)	16.4 (8.2-33.4)	0.94
Histopathology			
Low grade glioma (LGG), n=4	0	4 (100)	----
Oligodendroglioma, n=2	0	2 (100)	
Choroid plexus, n=1	0	1 (100)	
Location of Tumor			
Frontal	10 (22.7)	3 (42.8)	0.72
Parietal	3 (6.8)	1 (14.3)	
Cerebellar	4 (9.1)	1 (14.3)	
Temperoparietal	2 (4.5)	2 (28.6)	
Insular	4(9.1)	0	
Corpus Collosum	3(6.8)	0	
Temporal	11(25)	0	
others	7(15.9)	0	
Position/Laterality			
Left	26 (59.1)	4 (57.1)	0.38
Right	12 (27.3)	3 (42.9)	
Bilateral	1(2.3)	0	
Degree of Resection			
Gross total resection (GTR)	29 (65.9)	0	0.003
Near total resection (NTR)	11 (25)	4 (57.1)	
Subtotal resection	4 (9.1)	3 (42.9)	

visualization according to the identified histopathology, in which a bright, patchy, and homogeneous pattern was observed in all enrolled patients with various tumor histopathological types, except for low-grade glioma (LGG), oligodendroglioma, and choroid plexus papilloma.

Discussion

The results of this prospective study provided valuable insights into the use of sodium fluorescence-guided surgery in neurosurgical procedures for brain tumor resection. This technique has gained popularity for improving tumor resection and enhancing surgical outcomes. The prospective study had a diverse group of patients ranging from 19 to 77 years of age, indicating that brain tumors can affect individuals across a wide age range and with female predominance, and this was in contrast with an Indian study of a sufficient sample.[17,18] This may be due to low sample sizes. The tumors were observed in various brain regions, including the frontal, temporal, insular, parietal, cerebellar, and corpus callosum. Tumor distribution showed greater involvement of the left hemisphere than the right, with bilateral involvement in some cases. This suggests that brain tumors can affect either side of the brain with a preference.

Glioblastoma (GBM) (grade IV), astrocytic tumors (grades I–III), oligodendroglioma (grades II–III), and ependymomas (grades I–III) were the most prevalent histologic categories in adult patients.[19] Our study patients also commonly showed glioblastoma histopathology followed by astrocytic tumors.

This prospective study analyzed a diverse range of brain

Table 3. Sodium Fluorescein guided tumor pattern visualization according to the identified histopathology (N-51).

Histopathology types	Tumor Fluorescence Pattern	n (%)
Glioblastoma (GBM)	Bright patchy	19 (37%)
Metastasis	Bright patchy	6 (11.8)
Diffuse astrocytoma	Bright homo	5 (9.8)
Low grade glioma (LGG)	Dull patchy	4 (7.8)
Hemangioblastoma	Bright cyst wall	3 (5.8)
Anaplastic meningioma	Bright homo	2 (3.9)
Oligodendroglioma	Dull patchy	2 (3.9)
Lymphoma	Bright homo	2 (3.9)
High grade glioma (HGG)	Bright homo	2 (3.9)
DNET	Dull patchy	1 (2)
Choroid plexus papilloma	Unhelpful	1 (2)
Schwanomma	Bright homo	1 (2)
Myxopapillary ependymoma	Bright homo	1 (2)
Meningioma	Bright homo	1 (2)
Ependymoma	Bright homo	1 (2)

tumor cases, including various tumor locations and histopathologies. The majority of cases demonstrated positive fluorescence, indicating the presence of fluorescent tissue within the tumors. This suggests that fluorescence can serve as a reliable tool for intraoperative visualization and tumor boundary identification.

The fluorescence patterns observed were predominantly bright and homogeneous in three-fourths of cases, which aligns with previous studies[20-23] demonstrating that high-grade gliomas tend to exhibit intense and uniform fluorescence. This finding supports the notion that fluorescence can aid in distinguishing tumor from normal brain tissue, thereby facilitating more precise and complete resections.

In addition to overall fluorescence, patchy patterns were observed in some cases, indicating tumor heterogeneity. This finding underscores the challenges of achieving complete resection when fluorescent tissue is not uniformly distributed. Surgeons must carefully interpret and navigate these fluorescence variations to optimize the extent of resection.

The dataset also highlights the utility of fluorescence in assessing tumor resection: our research shows that surgery guided by sodium fluorescein improves the extent of tumor resection. Neurosurgeons can perform more accurate and thorough resections when they can differentiate tumor boundaries using positive fluorescence, especially in high-grade gliomas. Intraoperative Visualization: Using sodium fluorescein, surgeons can visualize and remove tumor sites that are not visible under white-light microscopy in real time. This is especially helpful when tumor heterogeneity is present. Gross Total Resection (GTR): The study highlights the effectiveness of sodium fluorescein in optimizing tumor removal, demonstrating that it facilitates GTR in a significant proportion of cases in which fluorescence is useful. Gross total resection (GTR) was achieved in more than 60% of cases in which fluorescence was helpful, indicating that fluorescence-guided surgery can effectively assist in achieving maximal tumor removal. However, cases with near-total or subtotal resection suggest that fluorescence alone may not ensure complete tumor removal, and other factors, such as tumor location and infiltration into critical structures, should also be considered.

It is worth noting that the dataset includes cases in which fluorescence was unhelpful or not used. This could be attributed to various factors, including tumor histopathology, timing and dosage of dye administration, or limitations in fluorescence detection.[24] Further investigations are necessary to better understand the factors influencing the effectiveness of fluorescence and to refine the technique for improved surgical outcomes. The sample size was too small to predict the usefulness of sodium fluorescein-guided tumor resection in other tumor types.

While this dataset provides valuable insights into the use of fluorescence in brain tumor resection, it is essential to acknowledge certain limitations, such as Tumor Heterogeneity: In some cases, patchy fluorescence patterns highlight tumor heterogeneity, making total excision difficult. To optimize the extent of resection, surgeons need to account for these variances and Variable Effectiveness carefully: the

dataset includes instances in which fluorescence was either ineffective or not used at all. The method's efficacy may be affected by variables such as time, dye dosage, tumor histology, and constraints on fluorescence detection. The dataset represents a specific set of cases and may not capture the full spectrum of brain tumors or surgical scenarios. Additionally, the dataset lacks detailed information on patient outcomes, including postoperative survival rates and functional outcomes. Sodium fluorescein can be a valuable tool for guiding surgical decision-making, particularly in achieving GTR. Its use could be especially advantageous during procedures where maintaining functional integrity is essential. Better Tumor Visualization: The method's ability to produce uniform, intense fluorescence patterns helps distinguish tumors from healthy brain tissue. This could aid surgery by improving visualization and identification of the tumor's borders. Additional Research and Technique Improvement: Our study highlights the need for further research to refine the variables that determine fluorescence effectiveness and to refine the technique to achieve better surgical outcomes. This creates opportunities for additional research and development in sodium fluorescein-guided resection.

A selection of papers investigating the use of sodium fluorescein-guided surgery for brain tumor removal is presented in the literature review. Acerbi et al. (2013) offered initial proof of possible advantages in grade IV gliomas, and their multicentric study from 2018, along with studies by Koc et al. (2008), Cho et al. (2019), and Schebesch et al. (2013), showed that sodium fluorescein-guided surgery is feasible and effective, especially for high-grade gliomas. Feasibility studies by Widhalm et al. (2013) and Schebesch et al. (2013) demonstrated the benefits of sodium fluorescein-guided resection using a specific surgical microscope filter. Through a randomized controlled experiment, Stummer et al. (2006) provided important insights by demonstrating the increased resection rate of malignant gliomas with 5-aminolevulinic acid-induced fluorescence-guided surgery. Taken together, these studies support sodium fluorescein's growing reputation as an essential intraoperative tool and demonstrate that it may improve surgical outcomes and resection rates in high-grade gliomas. A growing body of evidence supporting the practical relevance of sodium fluorescein-guided surgery in neurosurgical procedures is strengthened by the diversity of findings across numerous study designs and clinical contexts.

Conclusion

In conclusion, a modest dose of sodium fluorescein (3-5 mg/kg) can be used to detect and achieve gross and near-total resection of tumoral tissue effectively when utilizing a recently designed microscope integrated YELLOW 560 module (Carl Zeiss Meditec, Oberkochen, Germany) in certain tumors (GBM, HGG, and metastatic tumors).

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Ethical consideration

The study was approved by the Institutional Ethics Committee, IPGME&R, with the ethical approval code IPGME&R/IEC/2022/214.

Competing Interests

The authors declare no conflict of interest.

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