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Original Article

Clinical utility of serum glial fibrillary acidic protein in glial neoplasm

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ABSTRACT

Background: Glial fibrillary acidic protein (GFAP) is a member of the cytoskeletal protein family and is widely expressed in astroglial and neural stem cells, also in glial tumors such as astrocytoma and Glioblastoma (GBM). Increased GFAP expression and disruption of the blood–brain barrier are the characteristic features of GBM. Higher serum GFAP levels can help differentiate GBM from GBM mimics (such as primary central nervous system lymphoma, metastasis, or demyelinating lesions).

Methods: This prospective study was carried out in a tertiary care center in the department of neurosurgery on newly diagnosed glioma patients who underwent surgery from January 2018 to July 2019, excluded patients with history of the previous surgery for glioma, traumatic brain injury, and ischemic or hemorrhagic stroke. The blood sample was obtained at admission before undergoing invasive procedure. Pathological examination of the tumor biopsy sample was carried out using classical hematoxylin-eosin and immunohistochemical staining. All statistical analyses were performed using SPSS version 24.0.

Results: The mean preoperative tumor volume was 40 cm^3 (range 17.19–65.57 cm^3; standard deviation [SD] = 9.99 cm^3) which showed 98.25% mean reduction in volume postsurgery (mean tumor volume = 0.7 cm^3; SD = 0.19 cm^3). Preoperative serum GFAP measurements show higher levels (spearman's rho coefficient = 0.610 with P = 0.000) with increasing grade of tumor. GFAP levels also demonstrated higher value with increasing preoperative tumor volume.

Conclusion: Increasing serum GFAP levels in the preoperative period correlate with higher tumor grade, especially grade III and grade IV tumors. The serum GFAP levels showed relation to tumor volume, both before and after surgery.

Keywords: Central Nervous System(CNS), Glial fibrillary acidic protein (GFAP), Glioblastoma (GBM), Magnetic Resonance Imaging (MRI)

INTRODUCTION

Eng *et al.* first described glial fibrillary acidic protein (GFAP), a member of the cytoskeletal protein family and have widely expressed in astroglial and neural stem cells, also expressed in glial tumors such as astrocytoma and glioblastoma (GBM).[4] By adjusting the filament network present in the cell, astrocyte-neuron communication, and repair after central nervous system (CNS) injury, specifically for its role in forming glial scars throughout the CNS, GFAP plays an important role in mitosis.

GFAP is highly specific for cells with astrocytic differentiation and is widely used as a reliable marker in the immunohistochemical diagnosis and differentiation of brain tumors. Serum GFAP levels are also elevated in cases of brain trauma or hemorrhagic stroke.[2,3] Since GBM is characterized by increased GFAP expression and disruption of the blood–brain barrier, it is associated with increased serum levels of GFAP. Jung *et al*. and Tichy *et al*., in their study, concluded that serum GFAP constitutes a diagnostic biomarker for GBM.[6,8,12] Higher serum GFAP levels can help differentiate GBM from GBM mimics (such as metastasis, primary CNS lymphoma, or demyelinating lesions). Some studies have found a positive correlation between serum GFAP levels and tumor volume as well as tumor necrosis volume in the case of GBM.[8,9]

With the majority of studies concentrating on correlation between GBM and serum GFAP level, GFAP also has a potential role in largely unexplored areas such as monitoring therapy, detecting recurrence, and predicting the prognosis.[13] This study is designed to measure serum levels of GFAP in various grades of glioma and its correlation with tumor volume and further explore the postoperative trend in GFAP level and its relation with tumor progression in the Indian population.

MATERIALS AND METHODS

This prospective study was carried out in a tertiary care center in the department of neurosurgery on newly diagnosed glioma patients who underwent surgery from January 2018 to July 2019 after approval of the Institutional Ethical Committee. This study excluded patients with a history of the previous surgery for glioma, prior traumatic brain injury, and concomitant ischemic or hemorrhagic stroke.

The first blood sample was obtained at admission before undergoing any invasive diagnostic or therapeutic procedure. The second blood sample was obtained on the 7th postoperative day and the third at 3 months after surgery. Serum samples were centrifuged immediately in the laboratory, and supernatants were stored at −70°C for serum GFAP level measurement. Serum GFAP level was determined using a biotin-labeled antibody-based sandwich enzyme immunoassay for the quantitative measurement of GFAP. The total assay time was about 5 h and results were expressed as ng/mL.

Presurgical MR imaging of brain including standard sequences (T1-weighted before and after contrast, T2-weighted and fluid-attenuated inversion recovery sequence) was performed. The total tumor volume was estimated from preoperative MRI brain images using the modified ellipsoid formula (A × B × C)/2 with A, B, and C representing the maximum dimensions of the tumor or the necrotic area within the three-perpendicular axis. Residual tumor volume was evaluated using an MRI done within 48 h of surgery. Clinical evaluation and MRI brain were again repeated 3 months after surgery.

Pathological examination of the tumor biopsy sample was carried out using classical hematoxylin-eosin and immunohistochemical staining (including GFAP). Immunohistochemical staining of tumors for GFAP expression was ranked as <25%, 25–50%, 51–75%, and >75% GFAP-positive tumor cells.

The study data were collected and compiled in Microsoft Excel. All statistical analyses were performed using SPSS version 24.0 (IBM, Armonk, New York, USA). The categorical variables are presented as numbers and percentages, and the continuous variables were described as mean values, medians, and standard deviations (SDs). Paired *t*-test was used for univariate analysis and correlation was calculated using the Spearman correlation coefficient. ROC curves were used to analyze the cutoff value for serum GFAP.

RESULTS

Fifty patients (mean age: 39.5 years, 12 females) diagnosed with glial tumors were enrolled in this study. Headache (n = 28; 56%) was the most common symptom, followed by seizure (n = 20) and memory loss. The mean time of presentation from symptom onset was 7.1 months. The most common tumor subgroup was WHO grade III (Anaplastic astrocytoma, and anaplastic oligodendroglioma; n = 19) followed by the WHO grade IV tumors (GBM; n = 15) [Table 1].

The GBM subgroup presented at older age (GBM: mean age = 54.8 years; non-GBM: mean age 35.2 years vs.; P = 0.000) with seizure (n = 19) was the most common presenting complaint in this group.

The mean preoperative tumor volume was 40 cm^3 (range 17.19–65.57 cm^3; SD 9.99 cm^3) which showed 98.25% mean reduction in volume postsurgery (mean tumor volume = 0.7 cm^3; SD = 0.19 cm^3). However, at the end of 3-month follow-up period, mean tumor volume increased to 5.6 cm^3 (SD = 2.07 cm^3, range = 1.06–9.47 cm^3). The increase in volume was more significant in the GBM (mean = 0.06, CI = 0.06–0.07) group compared to the non-GBM group (mean = 0.04 cm^3, CI = 0.03–0.05) (P = 0.013). The immunohistochemical staining of tumor using GFAP showed 25–50% immunostaining in 50% of the cases with 28% showing <25% staining. The mean preoperative serum GFAP level was 0.046 ng/mL (range = 0.01–0.15; SD = 0.024) which remained largely unchanged in the immediate postoperative period (mean = 0.044 ng/mL; range = 0.02–0.15; SD = 0.027; P = 0.691) and at 3-month interval (mean = 0.043 ng/mL; range = 0.02–0.24; SD = 0.036; P = 0.809) [Figure 1].

Table 1: Tumor characteristics and distribution according to the WHO grading of glial neoplasm.

Factors			Frequency mean (SD)
Serum GFAP levels (as per tumor grade) ng/mL	I (*n*=4)	Preop	0.014 (0.009)
	• Pilocytic astrocytoma -Ganglioglioma	Postop	0.09 (0.058)
		3 m-post op	0.035 (0.006)
	II (*n*=12)	Preop	0.038 (0.006)
	• Diffuse astrocytoma	Postop	0.039 (0.003)
	• -Oligodendroglioma	3m-post op	0.033 (0.009)
	III (*n*=19)	Preop	0.044 (0.029)
	• Anaplastic astrocytoma	Postop	0.044 (0.029)
	• Anaplastic Oligodendroglioma	3m-post op	0.063 (0.054)
	IV (*n*=15)	Preop	0.064 (0.017)
	• Glioblastoma	Postop	0.038 (0.013)
		3m-post op	0.03
Tumor volume preop (cm^3)			40.0 (10.0)
Tumor volume postop (cm^3)			0.7 (0.2)
Tumor volume 3 months (cm^3)			5.6 (2.1)

GFAP: Glial fibrillary acidic protein, SD: Standard deviation

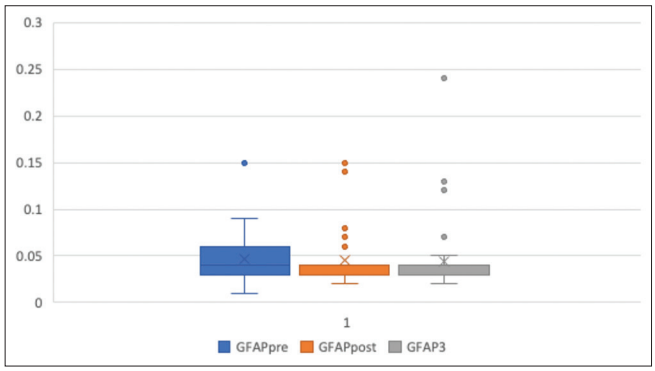

Figure 1: Serial variation in serum glial fibrillary acidic protein levels from preoperative period to immediate and 3-month postoperative period.

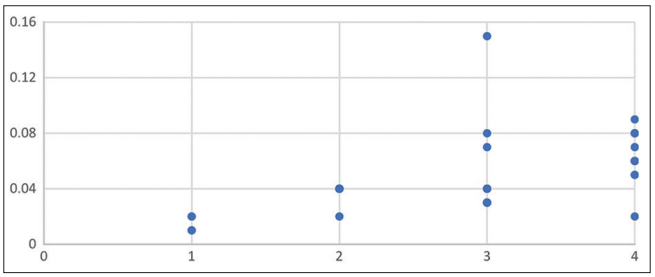

Figure 2: Relation of serum glial fibrillary acidic protein levels with the WHO grade I–IV of glial neoplasm.

Preoperative serum GFAP measurements show higher levels (spearman's rho coefficient = 0.610 with P = 0.000) with increasing grade of tumor, as shown in Figure 2. Moreover, preoperative GFAP levels also demonstrated a higher value with increasing preoperative tumor volume [Figure 3a], but it did not reach statistical significance (spearman's rho coefficient = 0.146 with P = 0.31). Serum GFAP levels in the immediate postoperative period (Spearman's rho coefficient = 0.306; P = 0.031) as well as 3-month interval (Spearman's rho coefficient = 0.384; P = 0.006) show positive correlation with the respective tumor volume [Figures 3b and c]. However, no correlation was observed between the change in tumor volume at 3 months with the serial change in serum GFAP levels (rho coefficient = 0.116; P = 0.423).

On subgroup analysis, immediate postoperative GFAP levels (0.038 ng/mL) showed a 44.92% decrease from the preoperative level of 0.069 ng/mL which, further, reduced at 3-month interval (0.03 ng/mL; P = 0.028). In the non-GBM group, the serum GFAP level demonstrated an unexpected increase from 0.039 ng/mL to 0.048 ng/mL in the immediate postoperative period [Table 2]. The mean preoperative tumor volume in both the groups was similar (P = 0.738), but there was a significant difference in the preoperative GFAP levels among them (P = 0.000). Using a ROC curve, the value of preop serum GFAP levels which corresponded with the diagnosis of GBM was determined to be 0.05 ng/mL (sensitivity = 0.933; 1-specificity = 0.086; area under curve (AUC) = 0.877) [Figure 4].

DISCUSSION

This study found that serum GFAP levels are elevated in patients with a diagnosis of GBM and these levels are demonstrably higher than that in grade II or grade III tumors (GFAP-positive cases: GBM-93.3% vs. non-GBM-8.5%; P = 0.00). In the previous studies, it is suggested that increased astroglial turnover and damage to blood–brain barrier are the dominant mechanisms responsible for elevated GFAP serum levels in GBM population compared to increased GFAP

Table 2: GBM versus non-GBM gliomas.

Category	GBM mean (SD)	Other gliomas mean (SD)	*P*
GFAP preoperative	0.069 (0.013)	0.039 (0.023)	**<0.001**
GFAP postoperative (7th day)	0.038 (0.03)	0.048 (0.03)	**0.034**
GFAP 3 month	0.03 (0)	0.047 (0.04)	**0.007**
Tumor volume: preoperative period (cm^3)	40.9 (8)	39.8 (10.6)	0.738
Tumor volume: immediate postoperative period (cm^3)	0.6 (0.1)	0.7 (0.2)	0.33
Tumor volume: 3-month follow-up (cm^3)	4.3 (1.5)	6 (2.1)	0.061

GBM: Glioblastoma, GFAP: Glial fibrillary acidic protein, SD: Standard deviation. Bold: $P < 0.05$ shows that GFAP has statistically significant relation to Glioblastoma in comparision with other glial tumors.

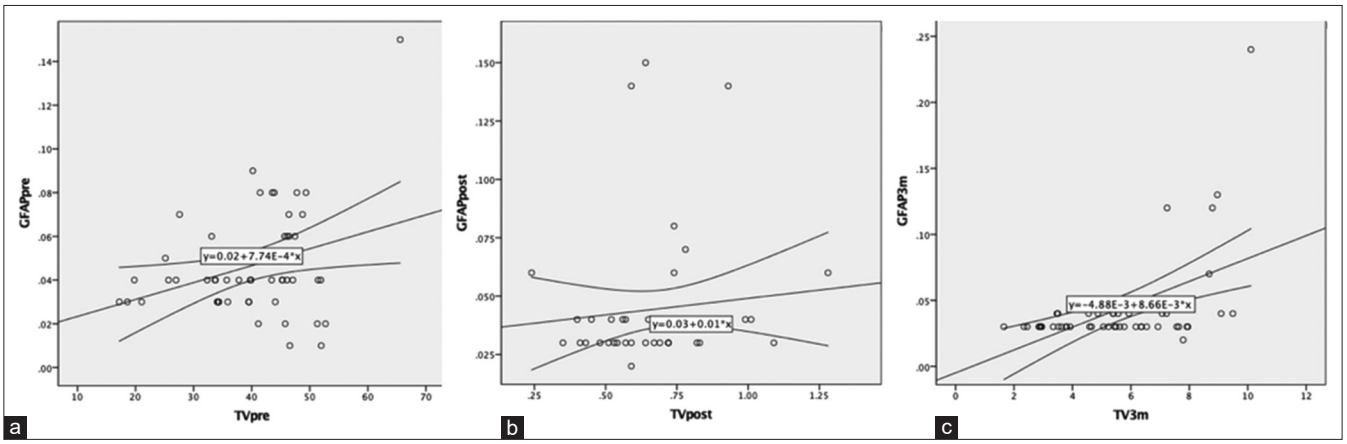

Figure 3: (a) Scatter plot showing relation of preoperative serum glial fibrillary acidic protein (GFAP) levels with preoperatively tumor volume, (b) scatter plot showing relation of postoperative serum GFAP levels with immediate postoperative tumor volume, and (c) scatter plot showing relation of serum GFAP levels and tumor volume at 3-month postoperative follow-up.

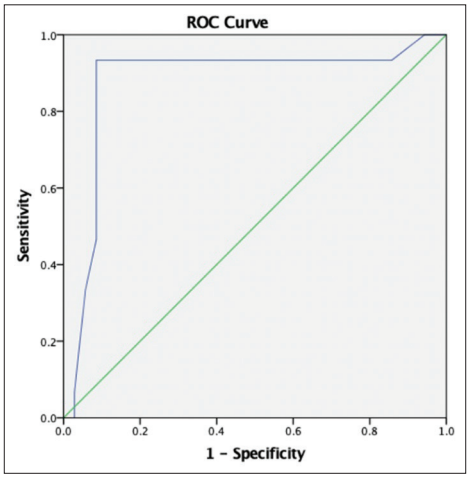

Figure 4: Receiver operating characteristic (ROC) curve showing the cutoff level for serum glial fibrillary acidic protein level for glioblastoma.

expression by tumor cells. This is, further, substantiated by the fact that cellular GFAP expression does not significantly differ between normal brain and GBM samples.[5] However, the rise in serum GFAP level is not consistently seen in all GBM cases.

Serial variations in serum GFAP level

Overall serum GFAP levels did not show a marked variation between preoperative to immediate postoperative period (0.046–0.044 ng/mL; $P = 0.691$) to 3-month interval (0.044–0.043 ng/mL; $P = 0.809$). However, on subgroup analysis, the GBM group showed a significant drop in serum GFAP levels postoperatively (0.069–0.038 ng/mL; $P = 0.000$) which demonstrated further decrease at 3-month follow-up (0.038–0.030 ng/mL; $P = 0.028$). Viietheer and colleagues found reduced serum GFAP levels compared to baseline at 6 weeks postsurgery which is similar to our observationsl.[1,11,13] Some studies have shown elevated serum GFAP levels up to 7 days postsurgery in the GBM population probably due to cell death subsequent to brain damage in the tumor and peri-tumoral zone resulting from surgical trauma.[1,9] On the contrary, the non-GBM group showed 23.07% increase in GFAP levels post-resection (0.039–0.048 ng/mL; $P = 0.008$). Other studies have failed to find any increase in the serum GFAP levels in the non-GBM tumor after resection.[5,13] The authors postulate that this discordant finding can be due to damage to the blood–brain barrier due to surgical manipulation following which more GFAP can pass through into the systemic circulation.

Correlation of GFAP with tumor volume

A positive correlation was observed between the tumor volume and GFAP level in the immediate postoperative period (spearman's rho coefficient = 0.306; $P = 0.031$) as well as at a 3-month interval (spearman's rho coefficient = 0.384; $P = 0.006$). Other authors have also found a positive correlation between serum GFAP levels with tumor volume.[8,9] However, studies by Baumgarten, Ilhan-mutlu, and Vietheer failed to find a correlation of GFAP with tumor volume.[7,10,13] The lack of correlation in other studies might be due to other parameters associated with detectable serum GFAP values such as GFAP expression, perfused blood volume, and edema formation which also influence serum levels. Although serum GFAP levels show a correlation with tumor volume postsurgery, the serial change in its level cannot be used as a predictor of tumor progression. However, this finding, further, raises an important question if there is a threshold phenomenon associated with this effect, that is, is there a threshold tumor volume only above which serum GFAP levels are elevated.

Variation of GFAP with grade

Our study revealed preoperative serum GFAP levels to correlate with tumor grade which is similar to the findings of Jung *et al*. who detected higher levels of serum GFAP levels in grade IV astrocytoma compared to other glial tumors.[8] Kiveniemi *et al*. reported higher serum GFAP levels in grade III astrocytoma as well, but the levels in grade IV tumors were significantly higher.[9]

GFAP in differentiating glioma from non-glial tumors

Significantly elevated serum GFAP levels were found in grade IV astrocytoma compared to grade II and III tumors, intracranial metastasis, as well as healthy controls.[5,8] This study showed serum GFAP levels of 0.05 ng/mL to be the most optimal cutoff value to differentiate GBM from lower grade glial tumors (sensitivity = 100%, specificity = 84.6%, positive predictive value = 81.8%, and negative predictive value = 84.6%). Jung *et al*. also reported GFAP level of 0.05 ng/mL to correspond with the diagnosis of GBM (sensitivity 76% and specificity 100%).[8] However, a lower threshold value (Tichy *et al*., GFAP cutoff value = 0.01 ng/mL) has also been reported which could be due to higher tumor volume and later presentation in our series.[12] Even though serum GFAP can be considered a diagnostic marker of GBM, the levels are not consistently raised in all cases of GBM. To explain this sensitivity gap, it has been suggested that the GFAP expression is lost during the evolution and dedifferentiation of malignant gliomas which might result in a decrease in their serum levels. The major limitations of this study are the relatively small sample size and short postoperative follow-up period. The role of adjuvant treatment has not been taken into consideration. The grading of tumors has not been done according to the latest genetic parameters as specified by the WHO brain tumor classification 2016. Long-term patient survival has not been assessed and correlated with serum GFAP levels.

CONCLUSION

Increasing serum GFAP levels in the preoperative period correlate with a higher tumor grade, especially grade III and grade IV tumors. The serum GFAP levels also showed a relation to tumor volume, both before and after surgery. A cutoff level of more than 0.05 ng/mL can be used to distinguish GBM from other glial tumors. However, serum GFAP levels could not predict residual tumor, nor did it show any relation to tumor progression, but further study with larger sample size is warranted to explore the role of any potential threshold phenomenon. This serum marker helps for the detection of glioma, particularly when there is a diagnostic dilemma on radiology. Moreover, the serum biomarker would save the patient from unneccessory radiation exposure in the follow-up to detect any recurrence and can differentiate radionecrosis from recurrence. The serum GFAP level will not be the replacement for the histopathology at confirmation of glioma in the beginning, however in recurrent glioma and for differentiation of radionecrosis, it would be of immense value. Once in routine practice, it will be much more cost effective than repeated radiology (CT/MRI scan), saving the patient from hassle of taking the patient to CT/MRI center, it will be of phenomenal comfort for bed ridden patient.

Declaration of patient consent

Patients' consent not required as patients' identities were not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

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