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Brain Tumor Vascularity Estimation by Arterial Spin Label Perfusion MRI—A Preoperative Tool for Patient Prognostication

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Abstract

Introduction Brain tumors remain a significant cause of morbidity and mortality around the globe. Preoperative estimation of tumor vascularity is of great significance for a neurosurgeon. Aim of our study was to correlate tumor blood flow (TBF) using arterial spin labeling perfusion imaging (ASL-PI) with microvessel density (MVD), tumor grade, and preoperative prognostication of brain tumors.

Materials and Methods This was a prospective observational cross-sectional study conducted in 63 patients of primary brain tumors already referred for magnetic resonance imaging. Absolute and relative mean and maximum TBF were calculated using ASL-PI and correlated with tumor grade and MVD at 10x and 40x magnificantion; thereby stydying the role of ASL-PI in brain tumor prognostication.

Results The mean of maxTBF values (mL/min/100 g) in the gliomas group, meningiomas group, schwannoma group, craniopharyngioma group, and hemangioblastoma group was 147.15, 251.55, 96.43, 43.3, and 578.3, respectively. The median of maxTBF value in the gliomas group, meningiomas group, schwannoma group, craniopharyngioma group, and hemangioblastoma group was 131.5, 158.63, 94.5, 43.4, and 578.3, respectively.

Discussion Significant correlation between meanTBF and MVD at 10X magnification (p-value < 0.001, rho =0.88) and a positive correlation between meanTBF and MVD at 40X magnification (p-value < 0.001) were seen. Significant correlation was also seen between maxTBF and MVD at 10X magnification (p-value < 0.001, rho = 0.91) and between maxTBF and MVD at 40X magnification. TBF in case of the hemangioblastoma was higher than other types of brain tumors (gliomas, meningiomas, and schwannomas). HighTBF value was seen in high-grade gliomas compared with low-grade gliomas with worse prognosis. TBF was high in typical meningiomas whereas low in atypical meningioma.

Conclusion TBF by ASL-PI can be considered a noninvasive in vivo marker in predicting the grade of brain tumors and further assist in envisaging prognosis of the patients with brain tumors.

Keywords
- Arterial spin labelled perfusion imaging (ASL-PI)
- brain tumors
- preoperative prognostication
- tumor blood flow (TBF) estimation
- tumor microvessel density

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Introduction

Brain tumor is an important cause of morbidity and mortality all across the world.[1] Digital angiography is considered as the ultimate gold standard for preoperative estimation of tumor vascularity, which is of great significance for a neurosurgeon; however, it is invasive and time consuming, hence not routinely done as part of tumor investigation protocol.[2,3] Magnetic resonance imaging (MRI) perfusion parameters give insight about the tumor vascularity and vessel proliferation in a reasonably noninvasive manner.[4–6] Conventional dynamic susceptibility contrast perfusion MRI (DSC) is a widely used technique in which initial transit of contrast medium is obtained using T2*-weighted images It involves bolus injection of contrast material through large bore intravenous cannulas for the evaluation of blood flow to the brain tumors. However, exogeneous contrast injection is not feasible in patients with poor renal status and generalized debilitated state.

Arterial spin labeling perfusion imaging (ASL-PI) is an evolving MRI modality that measures cerebral blood flow (CBF) and calculates CBF by magnetically labeling the arterial blood protons that flows into the region of interest (ROI), without the need of exogenous contrast. The difference in signal between the images is directly proportional to the extent of magnetization inversion received by the ROI. Consequently, ASL-PI is favorable for patients with renal dysfunction, pediatric population, and those necessitating repeated follow-up scans.[7]

There are hardly any studies evaluating relationship between ASL-PI estimated tumor vascularity, histopathological findings, and prognosis of brain tumor in Indian population. Therefore, there is a need to establish a correlation between ASL-PI derived tumor blood flow (TBF) and histopathological assessment of vascularity in brain tumors in our clinical scenario. Aim of our study was to correlate TBF using ASL-PI with microvessel density (MVD), tumor grade, and preoperative prognostication of brain tumors.

Materials and Methods

This was a prospective observational study in a tertiary care hospital over a period of eighteen months from September 2020 to February 2022. Total 63 patients of primary brain neoplasm already undergoing MRI were included in the study. Patients with a known primary tumor with cerebral metastases, previous brain tumor surgery, metallic clips, pacemakers, or other generalized contraindications for MRI were excluded.

Methodology

MRI was acquired in a single session on 3T MR Imaging Scanner GE Discovery, 750, Milwaukee, Wisconsin with a 32-channel phased array head-neck-spine coil. Prior approval was taken from our institutional ethics review board. Written informed consent was taken from every patient fulfilling the inclusion criteria. Relevant clinical history was obtained from all patient and findings were recorded in a pre-determined Proforma.

After performing conventional MRI sequences (pre- and post-contrast 3D T1-weighted (T1W), axial and coronal T2W and necessary fluid-attenuated inversion recovery images), 3D ASL was performed by the use of pseudo-continuous labeling technique. Ponto-medullary junction was used as the labeling plane to avoid the curved petrous segment of internal carotid artery (ICA) and select the terminal segment of ICA. The tumor in its greatest dimension was made to coincide with the imaging plane, by referring to the conventional MRI sequences.

Post-Processing

ASL-colored maps were produced using READY view software. ROIs were placed, in the axial plane, in the region with maximum perfusion based on the color maps corresponding to solid tumor as was seen on the T1W post-contrast image. Area with cysts, calcification, necrosis, and blood vessels were avoided (based on T1W and T2W images).

Three to five nonoverlapping equal sized ROIs individually measuring at least more than or equal to 5 mm were visually placed on the tumors in the axial color coded ASL images.

The absolute mean TBF (mean TBF) was calculated by averaging the value of 3 to 5 ROIs showing high TBF values. The absolute maximum TBF (max TBF) was represented by the ROI showing maximum value among all.

The mean CBF value (mean CBF) was calculated from 3 to 5 ASL ROIs placed in normal appearing cortical gray matter (using T1W images) and the maximum CBF value (maxCBF) was represented by the cortical ROI showing maximum value among all.

Values were normalized to CBF by calculating the ratio of absolute TBF values with CBF values to get relative TBF values (rTBFmean and rTBFmax).

Histopathological Evaluation

The patients were followed up and tumor tissue biopsy sample of operated patients was sent for histopathological analysis to look for MVD and tumor grade. To calculate the MVD, tissue sections were immunostained using a monoclonal mouse antibody directed against CD34 antigen, which identifies vascular endothelial cells. Areas with highest neovascularization (hot spots) were identified on low power magnification (40X) after CD34 immunohistochemistry was performed. Microvessels within the tumor were counted in three hotspots at 10X and 40X magnification and the mean of the three values was considered to be the MVD.

Statistical Analysis

Data was entered in MS EXCEL spreadsheet and analyzed using 21.0 version of Statistical Package for Social Sciences. Number and percentages represented categorical variables, while mean and median were represented by continuous variables. Normality of data was tested by appropriate statistical tests like Kolmogorov–Smirnov test. Correlation between perfusion parameters (meanTBF, maxTBF, rTBFmean, rTBFmax) and MVD (at 10X and 40X magnification) were performed using Spearman Rank Order Correlation.

Results and Observations

Total 63 patients of primary brain tumor were studied. The range for patient age was between 6 and 72 years and median age was 40 years. Forty-three (68.6%) males and 20 (31.4%) females were included. Most of the patients presented with complaints of headache, hemiparesis, seizures, and blurring of vision. There were 36 gliomas, 16 meningiomas, eight schwannomas, two craniopharyngiomas, and one hemangioblastoma. Data was normally distributed following a bell-shaped curve.

The mean of meanTBF values (mL/min/100 g) found in the gliomas group, meningiomas group, schwannoma group, craniopharyngioma group, and hemangioblastoma group was 145.58, 192.26, 85.77, 36.50, and 402.90, respectively. The median of meanTBF values in the glioma group, meningioma group, schwannoma group, craniopharyngioma group, and hemangioblastoma group was 121.65, 137.82, 83.5, 36.5, and 402.9, respectively.

The mean of maxTBF values (mL/min/100 g) in the glioma group, meningioma group, schwannoma group, craniopharyngioma group, and hemangioblastoma group was 147.15, 251.55, 96.43, 43.3, and 578.3, respectively. The median of maxTBF value in the glioma group, meningioma group, schwannoma group, craniopharyngioma group, and hemangioblastoma group was 131.5, 158.63, 94.5, 43.4, and 578.3, respectively.

The mean of rTBFmean values (mL/min/100 g) turned out to be 3.46 in case of glioma, 4.73 in case of meningioma, 2.22 in case of schwannoma, 1.17 in case of craniopharyngioma, and 14.92 in case of hemangioblastoma. The median of rTBFmean value was found to be 3.76 in glioma, 4.34 in meningioma, 2.49 in schwannoma, 1.17 in craniopharyngioma, and 14.92 in hemangioblastoma.

The mean of rTBFmax values (mL/min/100 g) turned out to be 4.12 in case of gliomas, 3.67 in case of meningiomas, 3.52 in case of schwannomas, 2.62 in case of craniopharyngiomas, and 3.87 in case of hemangioblastoma. The median of rTBFmax value was found to be 3.82 in gliomas, 3.53 in meningiomas, 5.1 in schwannomas, 3.6 in craniopharyngiomas, and 38.75 in hemangioblastoma.

The mean TBF, max TBF, rTBFmean, and rTBFmax value was maximum in hemangioblastomas followed by meningiomas and gliomas (►**Table 1**).

Positive correlation was observed between vessel count (at 10X/40X magnification) and meanTBF (mL/min/100 g), maxTBF (mL/min/100 g), rTBFmean, and rTBFmax value was statistically significant ($p = < 0.001$). The scatterplot (►**Fig. 1**) illustrates the association between rTBFmean/rTBF max with vessel count at 10X / 40X magnification, respectively.

The meanTBF, maxTBF, rTBFmean, and rTBFmax (mL/min/100 g) of gliomas was not normally distributed in different World Health Organization (WHO) grades. The median value of meanTBF in grade I, II, III, and IV gliomas (as per 2016 WHO classification) was 38, 71.7, 138.6, and 165.7 mL/min/100 g, respectively. Median value of TBFmax in increasing grades was 51, 78.2, 150, and 208 mL/min/100 g, respectively. However, no statically significant difference was observed (Kruskal–Wallis test) in mean TBF ($\chi2 = 4.588$, $p = 0.205$) among different subgroups. Strength of association (Kendall's Tau) came out to be 0.36 (medium effect size). Similarly no statically significant difference was observed in maxTBF ($\chi2 = 4.770$, $p = 0.189$) with strength of association (Kendall's Tau) being 0.37 (medium effect size), or rTBFmean ($\chi2 = 6.582$, $p = 0.087$) with strength of association (Kendall's Tau) as 0.48 (medium effect size). The rTBFmax ($\chi2 = 3.143$, $p = 0.370$) also showed no statically significant difference as strength of association (Kendall's Tau) was 0.12 (Small Effect Size).

TBF values in typical meningiomas were higher (TBFmean: 368.4 mL/min/100 g, TBFmax: 386.3 mL/min/100 g, rTBFmean: 6.5, rTBFmax: 52.9), and in a case of atypical meningioma TBF value was lower (TBFmean: 11.6 mL/min/100 g, TBFmax: 15 mL/min/100 g, rTBFmean: 0.4, and rTBFmax: 37.5).

Table 1 Tumor blood flow parameters in different tumor sybtypes

		Tumor type				
		Gliomas	Meningiomas	Schwannoma	Craniopharyngioma	Hemangioblastoma
meanTBF (mL/min/100 g)	Mean	145.58	192.26	85.77	36.5	402.90
	Median	121.65	137.82	83.5	36.5	402.9
	Range	25.8–297	8.3368.4	52–115	6–67	402.9–402.9
maxTBF (mL/min/100 g)	Mean	147.15	251.55	96.1	43.3	578.3
	Median	131.5	158.63	94.5	43.4	578.3
	Range	30.4–308.4	11–386.3	63–129.2	8.8–78	578.3–578.3
rTBF mean (mL/min/100 g)	Mean	3.46	4.73	2.22	1.17	14.92
	Median	3.76	4.34	2.49	1.17	14.92
	Range	0.81–8.25	0.4–6.52	1.16–3.01	0.25–2.09	14.92–14.92
rTBFmax (mL/min/100 g)	Mean	4.120	3.670	4.852	3.623	38.75
	Median	3.822	3.534	5.19	3.623	38.75
	Range	15.88–60.77	17.49–59.25	31.4–54.16	35.2–37.25	38.75–38.75

Abbreviation: rTBF, relative tumor blood flow.

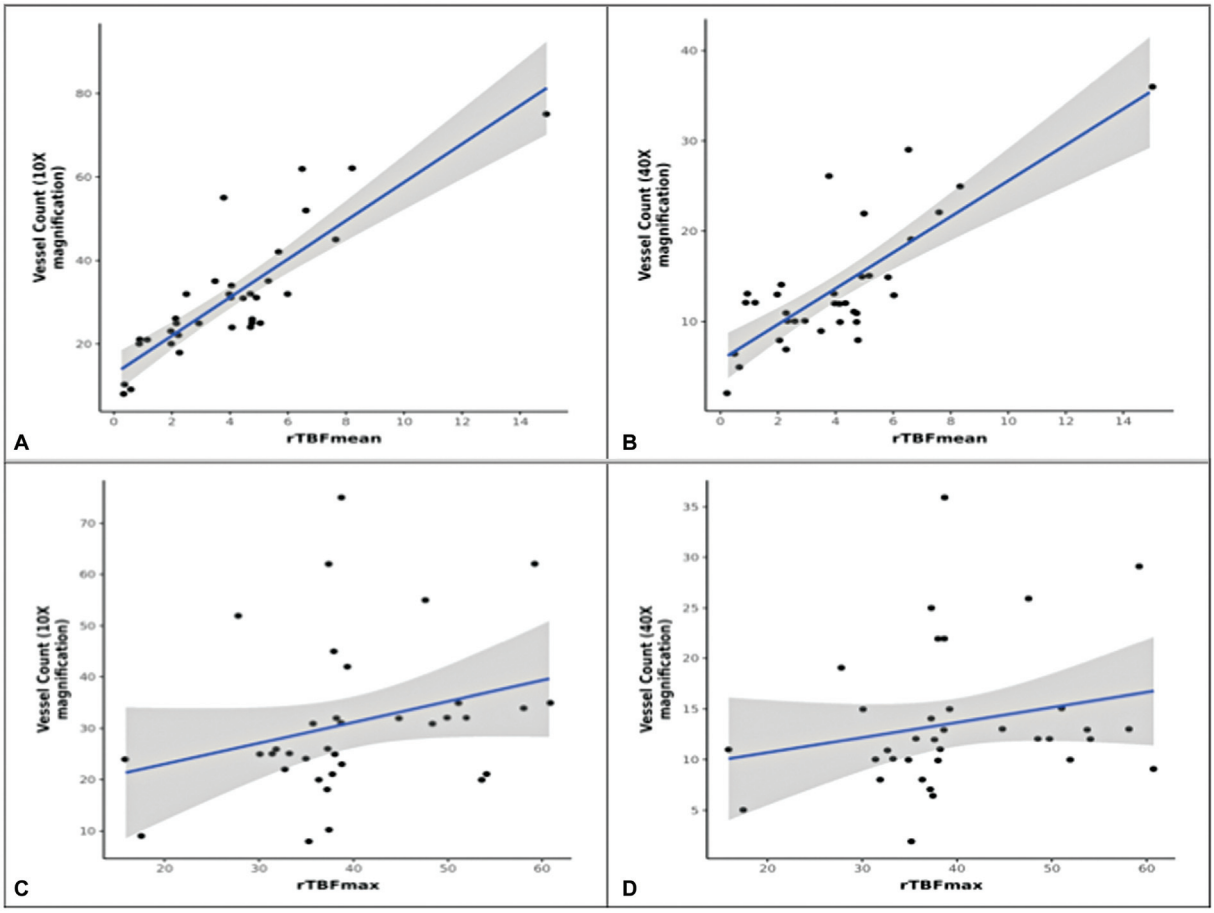

Fig. 1 Scatter plot diagram. Individual points represent individual cases. The blue trend line represents the general trend of correlation between the two variables. The shaded gray area represents the 95% confidence interval of this trend line. **A** and **B** depict correlation of relative tumor blood flow (rTBF) mean with 10X (**A**) and 40X (**B**) magnification. **C** and **D** depict correlation of rTBF max with 10X (**C**) and 40X (**D**) magnification. Spearman correlation coefficients were 0.8, 0.6, 0.4, and 0.3, respectively.

Discussion

ASL-PI, in which a magnetically "labeled" image is subtracted from a nonlabeled "reference" image can be used as a modality to estimate the MVD of tumor without any use of exogenous contrast, thereby predicting the vascularity of the tumor.

Our study showed a positive correlation among meanTBF and MVD at 10X magnification (p-value < 0.001, rho $= 0.88$) and a moderate positive correlation among meanTBF and MVD at 40X magnification (p-value < 0.001). Additionally, positive correlation was observed between maxTBF and MVD at 10X magnification (p-value < 0.001, rho $= 0.91$) and between maxTBF and MVD at 40X magnification. These findings were found to be in agreement with studies by Noguchi et al,[8] Koizumi et al,[9] Kikuchi K et al,[10] and Kimura et al[11] which also show a positive correlation between meanTBF and maxTBF with MVD (or percentage vessel count).

We found that TBF in case of the hemangioblastoma (►**Fig. 2**) was higher than other tumors (namely meningiomas, gliomas, and schwannomas), which was in agreement with the study performed by T. Noguchi et al.[8] This corroborates with the fact that there is marked neovascularity with large vessels within the lesion. This could potentially be helpful in eliminating the differential diagnosis of posterior fossa lesions having a cyst with solid nodule type of enhancement; the lesion with high TBF would point toward diagnosis of hemangioblastoma.

TBF by ASL-PI can assess tumor neovascularity, which plays a crucial role in histologic grading of tumor. Present study demonstrated low TBF values in low grade than high grade gliomas (►**Fig. 3**). In most of the cases, there was an increasing trend seen between TBF values and increasing grade of gliomas. Such association of TBF values with grading of gliomas was also demonstrated by Noguchi et al,[8] Wang et al,[12] Yeom et al,[13] and Abdel-Razek et al.[14] Arisawa et al in 2018 concluded that ASL-PI can be considered as an alternate perfusion MRI method in distinguishing low- to high-grade gliomas where DSC cannot be used.[15] Khashbat et al in 2017 established that TBF by ASL-PI can be used to distinguish low- to high-grade nonenhancing astrocytic tumors as well.[16] This study revealed a worse perioperative prognosis and a higher mortality in high as compared with low grade glioma.

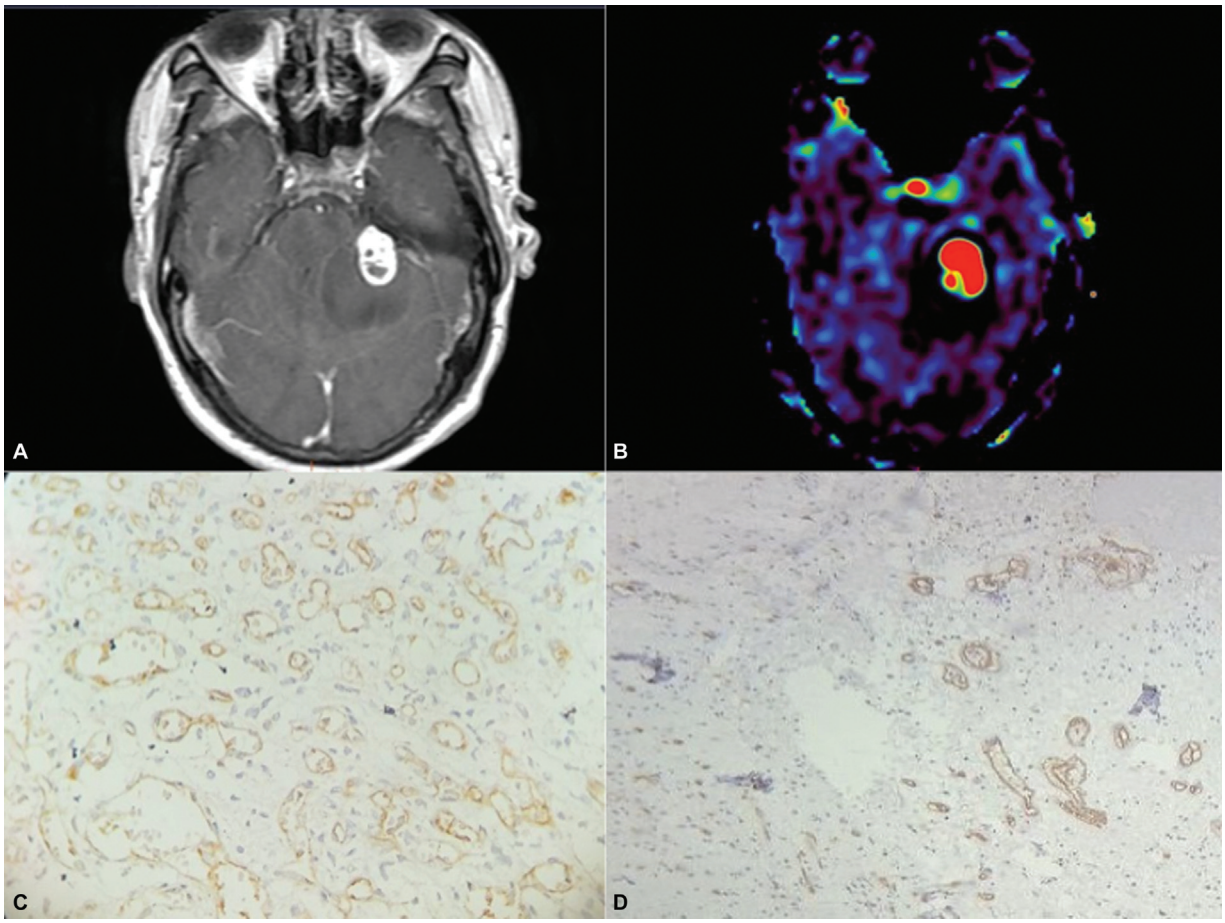

Fig. 2 Post-contrast axial T1-weighted imaging (**A**) and corresponding arterial spin labeling map (**B**) in case of a hemangioblastoma in posterior fossa show markedly raised tumor blood flow (TBF; TBFmean: 402.9 mL/min/100 g, TBFmax: 578.3 mL/min/100 g, rTBFmean: 14.9, rTBFmax: 38.7) in the enhancing areas. (**C** and **D**) Histopathological microvessel density seen on 10X and 40X magnification, respectively.

Our study uncovered that TBF was high in typical meningiomas whereas low in atypical meningioma (►**Fig. 4**). This was in concordance with study published by Qiao et al[17] who described three different visual patterns of ASL-derived CBF maps in cases of meningiomas with higher-grade meningiomas depicting no substantial hyper perfusion. This knowledge helped the surgeon as more difficulties were encountered in high grade/atypical meningiomas due to parenchymal invasion. There was better understanding of tumor margin and estimation of extent of resection preoperatively. Potentially complex surgeries with higher chances of complication and longer operative time could be predicted. As was seen in our case of angiomatous meningioma, where presence of pial-cortical arterial supply helped in predicting higher chances of bleeding and difficult resection. Similar findings were observed by ElBeheiry et al as well.[18]

In our study, ASL-PI proved valuable in estimating the absolute quantitative values of CBF. This is not always possible in DSC perfusion, as it lacks a direct linear relationship between signals changes and contrast concentration, more so in cases with partial volume artifacts. As ASL-PI relies on the intrinsic diffusible tracer, it was not affected by permeability characteristics in comparison to DSC perfusion MRI, in which permeability acts as a main confounding factor in the measurement accuracy of relative Cerebral blood Volume (CBV) values.[19] ASL helped in providing a roadmap for the evaluation of tumor infiltration. It was useful in targeting the site of biopsy from the highest-grade portion of the tumor, thus assisting accurate grading of a tumor.

Limitations

Our sample size was relatively small due to ongoing coronavirus disease 2019 pandemic at the time. Studies with a larger sample size and in different brain tumor subgroups can aid in establishing ASL-PI as a standard preoperative prognostic tool. Furthermore, ASL-PI is affected by the flow related biophysics, as inversion time for labeling varies with the changing hemodynamics. One needs to be careful while selecting these parameters, as low values are required for pediatric and geriatric populations. ASL-PI has low signal to noise ratio with longer acquisition time when compared with other perfusion techniques. Another limitation of ASL is that it can produce an

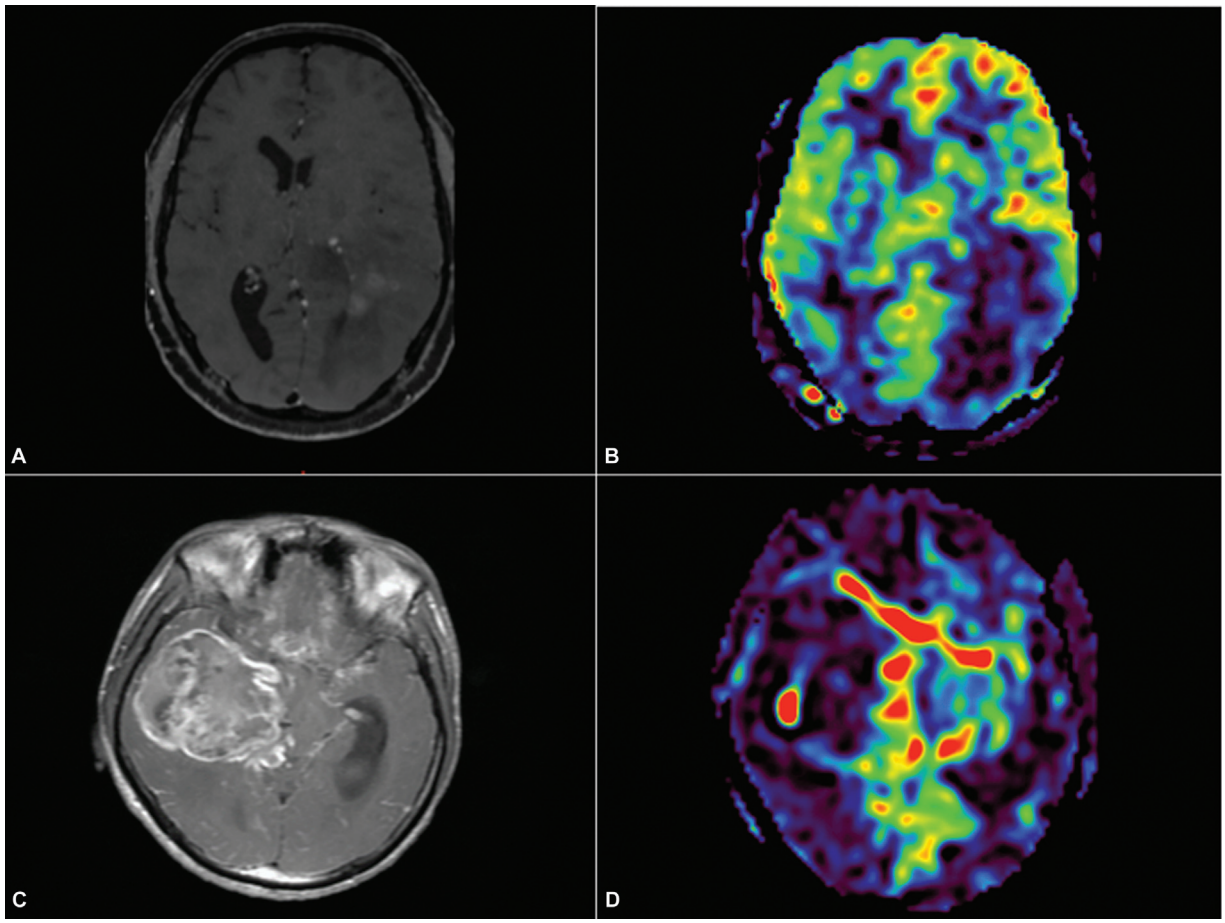

Fig. 3 Post-contrast axial T1-weighted imaging (**A, C**) and corresponding arterial spin labeling map (**B, D**) in case of a low grade (**A, B** with TBFmean: 67 mL/min/100 g, TBFmax: 75mL/min/100 g, rTBFmean: 4, rTBFmax: 58.3) and high grade (**C, D** with TBFmean: 195 mL/min/100 g, TBFmax: 266.3 mL/min/100 g, rTBFmean: 5.9, rTBFmax: 44.7) gliomas, respectively. rTBF mean, relative mean tumor blood flow.

underestimation of CBF by causing prolongation of arterial transit times causing relaxation of spin-label in cases of severe ischemia.[20] Some low-grade tumors may also exhibit excessive vascularity, thereby showing markedly raised TBF as in the cases of oligodendroglioma. In addition, presently ASL-PI can provide only CBF values; however, future developments in ASL techniques may be able to derive other perfusion parameters like CBV for brain tumors. Limited published literature could be found on grading and prognostication of brain tumors based on ASL-PI.

Conclusion

In our study, we observed a positive association between TBF calculated using ASL-PI and the brain tumor MVD, thereby assessing tumor vascularity. This knowledge plays an important role in surgical decision making, scheduling presurgical interventions such as preoperative embolization, and provides valuable acumen in predicting patient prognosis. TBF by ASL-PI can be considered a noninvasive in vivo marker in predicting the grade of brain tumors and further assist in envisaging prognosis of the patients with brain tumors. However, comprehensive investigation of correlation between ASL-PI, tumor vascularity, and grading is further necessary in specific subtypes of brain tumor. Further development is desired in ASL-PI technique to additionally assist in CBV calculations in assessing brain tumor vascularity.

Authors' Contribution

J.G. and R.J. contributed in conceptualization, reviewing, and editing of draft. N.R. collected data and formulated initial draft. B.P.S. provided surgical details and reviewed the draft. K.B.S. provided surgical details. S.Z. performed histopathological analysis.

Conflict of Interest

None declared.

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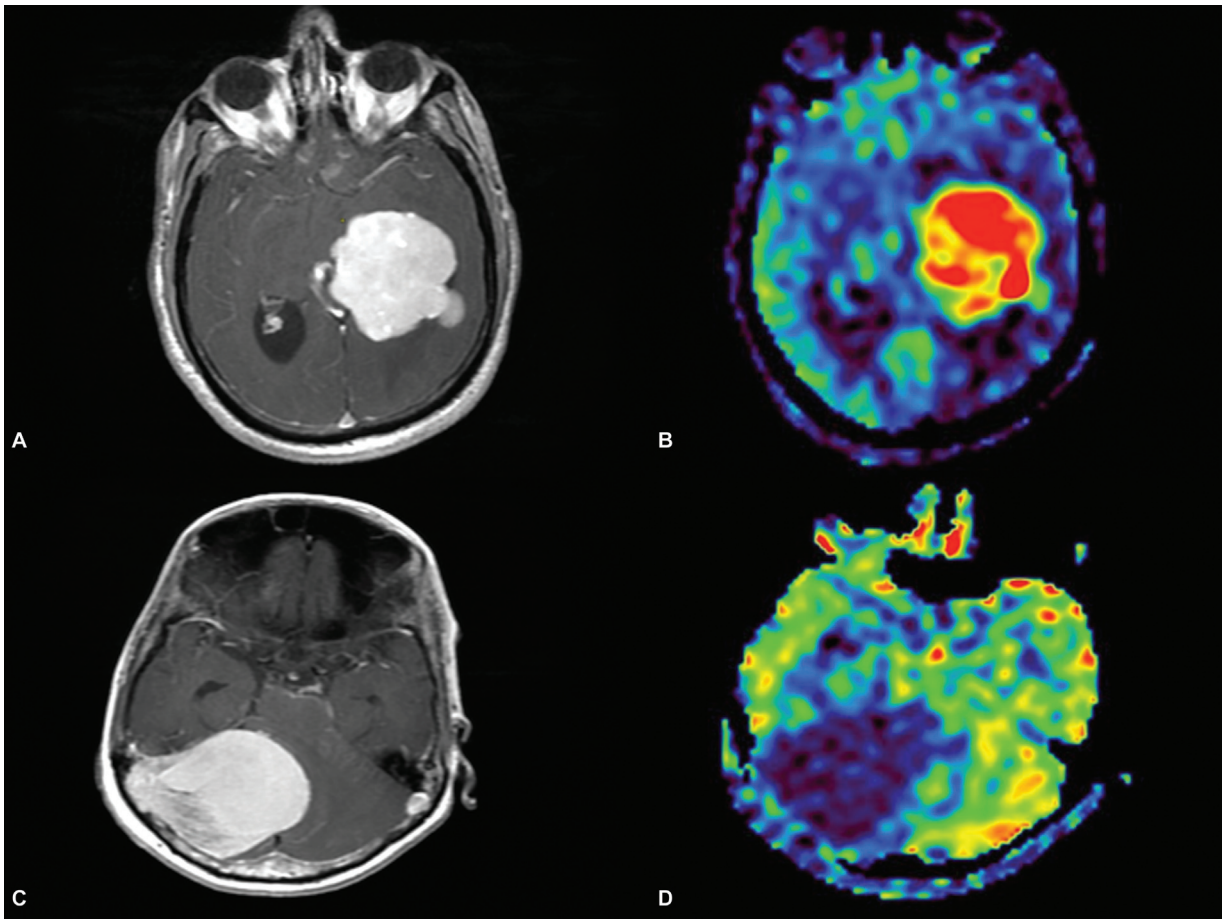

Fig. 4 Post-contrast axial T1-weighted imaging (**A, C**) and corresponding arterial spin labeling map (**B, D**) in case of typical (**A, B** with TBFmean: 127.6 mL/min/100 g, TBFmax: 143.6 mL/min/100 g, rTBFmean: 4, rTBFmax: 35.7) and atypical (**C, D** with TBFmean: 11.6 mL/min/100 g, TBFmax: 15 mL/min/100 g, rTBFmean: 0.4, rTBFmax: 37.5) meningiomas, respectively. rTBF mean, relative mean tumor blood flow.

kind contribution in data collection, patient management, and review of written article.

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