Discriminant Analysis to Classify the glioma grading using DCE MRI and immunohistochemical markers

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Introduction

Gliomas are the most common cerebral neoplasms. These are being graded according to World Health Organization classification from grade 1 to grade 4. Grade 1& 2 are considered as low grade while 3 & 4 as high grade gliomas[1]. Grading of glioma is of utmost clinical importance as it determines the appropriate therapy to be used for the treatment of the patient. The current gold standard for glioma grading is the histopathological assessment of excised tumor. The stereotactic biopsy is limited by the inherent small sample size. Though Gadolinium based conventional MR imaging is routinely used to predict the grade of glioma, it often misleads the clinician[2]. Perfusion MRI using dynamic susceptibility contrast and dynamic contrast enhanced methods have been widely implicated in assessment of glial neoplasm[3]. Both these perfusion imaging techniques have their merits and demerits but DCE seems to score over DSC in imaging those lesions which have a significant disruption of the blood brain barrier i.e. a significant leakage of contrast material. Matrix metalloproteinase (MMP)-9, phosphatase of regenerating liver (PRL)-3, hypoxia inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF) have been shown to express in varying degree among different grades of glioma. The study was aimed to pool the perfusion metrics and various immunohistochemical markers to see which ones are the best predictor of histological grade of these gliomas.

Materials and methods

***Subjects*:** Seventy six untreated patients (55 high grades & 21 low grades) with a post surgical diagnosis of glioma were included in this study.

***Data acquisition*:** All patients underwent preoperative imaging with both conventional and DCE-MRI on a 1.5 Tesla scanner (Echo-speed plus, General Electric, Milwaukee, USA) using a quadrature transmit–receive head coil. Institutional ethics and research committee approvals were obtained. DCE-MRI was performed using a three dimensional spoiled gradient recalled echo (3D-SPGR) sequence [TR/TE/flip angle/ number of excitation(NEX)/slice thickness/field of view (FOV)/matrix size=5.0ms/1.4ms/15°/0.5/6mm/360×270mm/128×128mm, number of phases=32]. At the fourth acquisition, Gd-DTPA-BMA (Omniscan, GE Healthcare, USA) was administered intravenously with the help of a power injector (Optistar^{TM} MR, Mallinckrodt, Liebel-Flarsheim, Ohio) at a rate of 5ml/sec, followed by a bolus injection of 30ml saline flush. A series of 384 images in 32 time points for 12 slices were acquired with a temporal resolution approximately of 5.25sec. Prior to 3D SPGR, fast spin echo (FSE) T_1-weighted (TR/TE/NEX/slice thickness/FOV/matrix size= 375ms/9.4ms/1/6mm/360×270mm/256×256mm) and fast double spin echo PD and T_2 weighted (TR/TE1/TE2/NEX/slice thickness/FOV/matrix size= 3500ms/25ms/85ms/1/6/360×270mm/256×256mm) imaging were performed for the same slice position to quantify voxel wise pre-contrast tissue T_{10}[4].

***MRI data processing and quantitative analysis*:** Voxel wise tissue T_{10} was calculated from FSE T_1, T_2 and PD images. The pharmacokinetic model was implemented for permeability (K^{trans}&K_{ep}) leakage (V_e) and plasma volume (V_p) calculations. Corrected CBV maps were generated by removing the leakage effect of the disrupted blood brain barrier[4]. For the calculation of perfusion metrics, a quantitative analysis of the concentration time curve was performed for calculation of cerebral blood volume (CBV) and cerebral blood flow (CBF). ROIs (40mm^2) were drawn on the region of tumor with the highest value of each perfusion metrics as seen by respective map of that metrics. A total of 5 slices on each metrics map were taken for placing ROIs where the lesion appeared to have the best values of respective perfusion metrics. Relative quantification of CBV (rCBV) and CBF (rCBF) were quantified by placing the ROI on normal contra-lateral grey/white matter of the brain.

Histopathology: The excised tumors were immuno-stained for monoclonal antibody against human MMP-9, VEGF, PRL-3 and HIF-1α antigen. Each immunostained slide was digitized with 10X objective using Canon Power Shot G5 camera and the captured images were subjected to morphometry analysis. The percentage of ten areas with maximal positive staining for MMP-9 was calculated at 10X resolution.

***Statistical analysis*:** To classify subjects into high and low grade tumor, information on 10 discreminatory variables was collected. Discriminant function analysis was used to identify discriminatory variables using a stepwise procedure. To study the relationship between immunohistochemical parameters and DCE metrics, Pearson's correlation coefficient was computed and tested for significance. A p-value ≤0.05 was considered as significant.

Results

Out of 10 variables considered for the analysis, four were found to be significant discriminators of the tumor grade (Table.1). Among these, HIF-1α was the only immunohistochemical marker and rest belongs to DCE metrics (CBV, Kep and Ve). Variables rCBV, Kep, Ve and HIF-1α were included to discriminate between low and high grade of tumor with canonical correlation 0.814. Discriminant coeffecients for these variables are presented in table1 and discriminnat functions for low (D_1) and high (D_2) grade tumor are given below.

$$D_1 = 0.499*CBV + 0.769*Kep + 0.061*HIF - 0.987*Ve \qquad D_2 = 1.266*CBV + 3.446*Kep + 6.731*Ve + 0.156*HIF$$

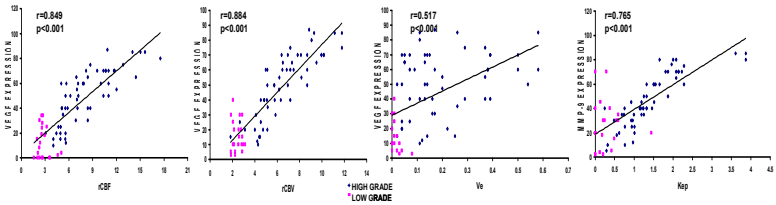

***Fig 1**: Showing significant positive correlation between 1) VEGF & rCBF 2) VEGF & rCBV 3) VEGF & V_e and 4) MMP-9 & K_{ep}*

Variables	Wilks' Lambda	Discriminant function coefficient		Mean ± S.D.
		Low grade	High grade	
rCBV	0.394	0.499	1.266	5.539±2.779
K_{ep}	0.442	0.769	3.446	1.137±0.869
V_e	0.363	-0.987	6.731	0.151±0.159
HIF	0.371	0.061	0.156	26.151±15.12

***Table.1:** Showing discriminant functions of variables*

These discriminant functions were tested for misclassification and fitness of the model to the actual data. A total of 7.9% cases reported were misclassified while 92.1% cases were correctly classified having a p value <0.001. On Pearson's correlation, VEGF expression correlated with rCBV, and rCBF, whereas MMP-9 expression correlated with K_{ep} (Fig.1). There was also a significant correlation between rCBV and HIF-1α expression irrespective of the grade of tumor.

Discussion

On discriminate analysis we found that among all the perfusion metrics, rCBV, Kep and Ve were able to differentiate between low grade and high grade glioma. The significant positive correlation of VEGF with rCBV and rCBF confirms these imaging parameters as the surrogate marker of its expression and hence neoangiogenesis. The correlation of VEGF & rCBV with HIF-1α indicate that both VEGF & HIF-1 α are related to each other in a same cascade of events that regulates neoangiogenesis. MMP-9 has been shown to be responsible for extracellular matrix degradation and in turn facilitates tumor cell migration and metastasis. The strong positive correlation of MMP-9 expression with Kep, irrespective of the grade of glioma suggests that it can be used as a surrogate of its expression and hence tumor progression and invasion. We conclude that DCE-perfusion MRI is a powerful tool to delineate glial neoplasm and can differentiate between high grade and low grade brain tumors which in turn is important in selection of correct management and treatment planning of these patients.

References

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