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. 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. Perfusion MRI using dynamic susceptibility contrast and dynamic contrast enhanced methods have been widely implicated in assessment of glial neoplasms. Both these perfusion imaging techniques have their merits and demerits but DCE seems to be the best predictor of histological grade of these gliomas. DCE-MRI was performed using a three dimensional spoiled gradient recalled echo (3D-SPGR) sequence [TR/TE/NEX/slice thickness/FOV/matrix size=5ms/1.4ms/15º/0.5/250×250/360×270×250mm, 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&trade; 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) T2-weighted (TR/TE/NEX/slice thickness/FOV/matrix size=375ms/9.4ms/1/600×360×270×256×256) and fast double spin echo PD and T2 weighted (TR/TE/TE2/NEX/slice thickness/FOV/matrix size=3500ms/25ms/16ms/250×270×256×256mm) imaging were performed for the same slice position to quantify voxel wise pre-contrast tissue T2. MRI data processing and quantitative analysis: Voxel wise tissue T2 was calculated from FSE T1, T2 and PD images. The pharmacokinetic model was implemented for permeability (K trans & Kep) and plasma volume (Vp) calculations. Corrected CBV maps were generated by removing the leakage effect of the disrupted blood brain barrier. 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*) were drawn on the region of tumor with the highest value of each perfusion metrics as seen by respective map of that metric. A total of 5 slices of each such 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 immunostained 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 discriminatory 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 coefficients for these variables are presented in table1 and discriminant functions for low (D1) and high (D2) grade tumor are given below.

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

These discriminant functions were tested for misclassification and fitness of the model to the actual data. A total of 7.9% 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 Kep (Fig.1). There was also a significant correlation between rCBV and HIF-1α expression irrespective of the grade of tumor.

Discussion

On discriminative 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 neangiogenesis. The correlation of VEGF & rCBV with HIF-1α indicate that both VEGF & HIF-1α are related to each other in a same cascade of events that regulates neangiogenesis. 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 neoplasms 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