Dynamic Contrast Enhanced Derived Cerebral Blood Volume Correlates Better with Leak Correction than With no Correction for Vascular Endothelial Growth Factor, Micro Vascular Density and Grading of Astrocytoma

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Introduction

Astrocytomas are the more commonly seen gliomas in adults and differs greatly in terms of their potential for growth, invasiveness, and tendency to progression.[1] Role of angiogenesis in development and aggressiveness of glioma has been well reported.[2] Perfusion magnetic resonance imaging (pMRI) has made it possible to assess the microvasculature in-vivo.[3] In this study, quantification of both hemodynamic and physiological indices in 64 cases of astrocytoma was done using dynamic contrast enhanced (DCE) MRI and correlated with immunohistochemically obtained microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression. The predictive value of various perfusion indices with and with out the correction for the leakage of contrast in grading of astrocytomas was also evaluated.

Materials and methods

***Subjects*:** Seventy four patients presented with sign and symptoms of mass lesion and/or seizures and were initially identified on conventional MRI. Out of 74 patients, 64 patients had astrocytoma while the remaining 10 patients had pure or mixed oligodendroglioma. The patients with oligodendroglioma were excluded from the study. The 64 patients (mean age±SD=35±9.54) with astrocytomas were classified based on WHO classification. There were 5 grade-I, 17 grade-II and 7 grade-III astrocytomas. The Grade-IV lesions included glioblastoma multiforme (n=34) and astrocytoma (n=1).

***Data acquisition*:** The informed consent was obtained before dynamic contrast enhanced (DCE) MRI. All patients underwent both conventional and DCE MRI on a 1.5 Tesla scanner (Echo-speed plus, General Electric, Milwaukee, USA) using quadrature transmit–receive head coil. The institutional ethical as well as the research committee approved the study protocol. DCE MR imaging 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/15º/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 weighted images.[4] Quantitative analysis of concentration time curve was performed for calculation of cerebral blood volume (CBV) and cerebral blood flow (CBF).[4] Pharmacokinetic model was implemented for permeability (k^{trans}) and leakage (v_e) calculation.[4] Corrected CBV map was generated by removing the leakage effect of the disrupted BBB.[4] For the calculation of perfusion indices a total of ten ROIs (20mm^2) were drawn on the region with the highest value as defined by the CBV color-coded map on each slice. Relative quantification of CBV (rCBV) and CBF (rCBF) was performed by placing the ROI on normal contra-lateral portion of the brain.

***Histopathology*:** All the excised astrocytomas were immuno-stained separately for polyclonal antibody against human VEGF (A-20) and monoclonal antibody to CD-34 antigen. Each CD-34 and VEGF immunostained slide was digitized with 40X objective using Canon Power Shot G5 camera and the captured images were subjected to morphometry analysis. To determine the MVD, all discrete, positively immunostained endothelial clusters with lumina were counted in ten 40X fields. The % number of positive VEGF cells were also counted from ten 40X fields.

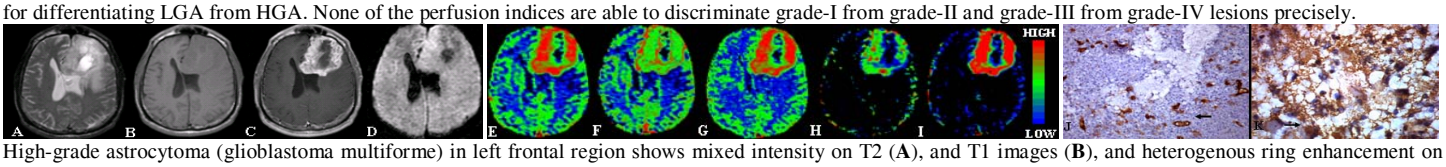
***Statistical analysis*:** Student t-test was performed to see the difference in perfusion indices, MVD and VEGF among different grades of astrocytoma. Pearson's correlation analysis between the perfusion indices, MVD and VEGF was performed. Spearman rank correlation was performed between various perfusion indices and astrocytoma grades. Discriminant analysis using rCBV and k^{trans} was performed to distinguish between the tumors grade (grade-I to IV).

Results

The grade-III and grade-IV astrocytoma showed significant increase in all perfusion indices with significant higher MVD and VEGF expression, compared to grade-I and grade-II astrocytoma. In grade-III, the corrected rCBV, rCBF, MVD and VEGF was significantly low compared to grade-IV astrocytoma while no significant change was seen in uncorrected rCBV, k^{trans} and v_e. In each grade of astrocytoma the corrected rCBV correlated significantly with MVD. The VEGF correlated significantly with corrected rCBV and MVD in astrocytoma which showed positive VEGF immunoreactivity. The corrected rCBV strongly and positively correlated with tumor grade (r=0.853, p=<0.001). The uncorrected rCBV (r=0.592, p=<0.001) and k^{trans} moderately (r=0.498, p=0.001) correlated with tumor grade. The corrected rCBV accurately discriminated 100% grade-I, 64.7% grade-II, 100% grade-III and 60.0% grade-IV astrocytoma. Using uncorrected rCBV and k^{trans} as a discriminant parameter the discriminate percentage in each group was further decreased. When grade-I and grade-II grouped as low-grade astrocytoma (LGA) and grade-III and grade-IV as high-grade astrocytoma (HGA), only the corrected rCBV for the leak discriminated LGA from HGA with 100% accuracy.

Discussion

It has been reported that high VEGF expression in enhancing tumor is associated with increased BBB permeability. Maia et al.[5] have reported high VEGF expression in non-enhancing HGA as well as LGA. In current study, non-enhancing HGA and LGA also showed positive VEGF expression. This finding forces to suspect the role of VEGF in long term increased BBB permeability. The up-regulation of vasoactive molecules i.e. bradykinin and leukotriene C4 in brain tumors has been reported in selectively increased capillary permeability.[6] We speculate that the expression of these molecules is probably responsible for the long term increased BBB permeability. Positive correlation between glioma grade and rCBV has been reported even in case of leaky BBB.[7] However, in all these studies there was overlapping in rCBV value between high-grade and low-grade glioma. We believe that the factor which may contribute to these overlapping is the non correction of leakage factor in rCBV quantification. As shown in this study, the corrected rCBV correlated strongly compared to uncorrected rCBV with astrocytoma grade. It has been reported that the rCBV is able to classify grade-I, grade-II and grade-III glioma separately based on the significant statistical difference. In this study, even when there was significant difference in corrected rCBV values among different astrocytoma grades the discriminant analysis showed that none of the perfusion indices either in isolation or in combination were able to separate all the four grades with 100% accuracy. Only the corrected rCBV was able to classify 100% HGA and 100% LGA. We conclude that, correlation among corrected rCBV, MVD and VEGF suggest that the corrected rCBV is a better in-vivo marker of angiogenesis. Corrected rCBV is more accurate for differentiating LGA from HGA. None of the perfusion indices are able to discriminate grade-I from grade-II and grade-III from grade-IV lesions precisely.



High-grade astrocytoma (glioblastoma multiforme) in left frontal region shows mixed intensity on T2 (**A**), and T1 images (**B**), and heterogenous ring enhancement on PCT1 image (**C**). The DWI image shows mixed intensity (**D**). The hemodynamic maps are showing increased uncorrected rCBV (7.65) (**E**), corrected rCBV (6.21) (**F**) and rCBF (6.67) (**G**) on the periphery of the tumor. Physiological maps [k^{trans} (1.29 min^{-1}) (**H**) and v_e (0.51) (**I**)] show the high permeability of contrast across the BBB. Microvessels enhance brown with CD34 immunostain (arrow) (**J**) and strong cytoplasmic VEGF expression by high percentage of tumor cells (arrow) (**K**).

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