DCE MRI derived Kep is a surrogate marker of MMP-9 expression in patients with Glioblastoma multiforme

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Introduction

The Blood brain barrier (BBB) is an effective physical barrier formed by capillary endothelial cells, pericytes and astroglial and perivascular macrophages. It is believed to be located at the inner endothelial walls of cerebral microvessels [1] and is responsible for the regulation of homeostasis in CNS. The integrity of BBB is challenged in various pathologies like infection and neoplasm.[2,3] Matrix metalloproteinases (MMPs) have been shown to be responsible for tissue destruction leading to opening of BBB.[4] Degradation of extracellular membrane and basement membrane contributes in the process of tumor progression and MMPs are particularly important for matrix degradation. Dynamic contrast enhanced (DCE) MRI metrics have been used to quantify the extent of BBB disruption.[5] These include, volume transfer coefficient (Ktrans), rate transfer constant between the extracellular extravascular space (EES) and the plasma (Kep) leakage (Ve) and plasma volume (Vp). This study, in patients with glioblastoma multiforme (GBM) explored correlation of DCE-MRI metrics with MMP-9 expression to identify whether it might be used as its surrogate.

Materials and methods

***Subjects*:** Seventeen previously untreated patients with a post surgical diagnosis of GBM 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/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}.[6] (ref)

***MRI data processing and quantitative analysis*:** Voxel wise tissue T_{10} was calculated from FSE T_1, T_2 and PD weighted images. The pharmacokinetic model was implemented for permeability (Ktrans and Kep) leakage (Ve) and plasma volume (Vp) calculations. Corrected CBV maps were generated by removing the leakage effect of the disrupted BBB.[6] 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 the tumor with the highest value of each perfusion metrics as seen by the respective map of that metrics (Fig.1). 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. The duration of survival of these patients (in months) was calculated from the date of surgery using the Kaplan-Meier method.

Histopathology*:* The excised GBMs were immuno-stained for monoclonal antibody against human MMP-9 (2C3, sc-21733, CA) antigen (Fig.1). Each MMP-9 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*:** Pearson's correlation was performed between all the perfusion metrics (obtained from the maximum value of single metrics at one time in 5 slices, representing the best visualization of the lesion) and immunohistochemically obtained percentage expression of MMP-9.

Results: All the perfusion metrics obtained were correlated with immunohistochemically obtained MMP-9 expression and with the duration (in months) of survival (Fig.2). Among all the perfusion metrics, only Kep was found to have a significant positive correlation with MMP-9 expression in all data sets no matter which perfusion metrics was considered for its maximum value during quantitation. We could only follow up 8 out of the 17 patients upto death as the rest were referred elsewhere for radiotherapy +/- chemotherapy. In these 8 patients, Kep also showed a significant negative correlation with the duration of survival. Except Vp, all the perfusion metrics (Kep, Ktrans, Ve, Vp, rCBV and rCBF) and also MMP9 expression showed a significant negative correlation (r=-0.724, p=0.042) with the duration of survival.

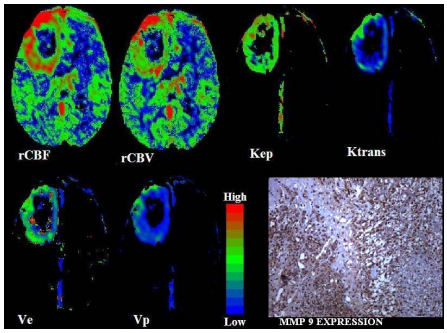

Fig 1: Color coded perfusion maps and immunuhistochemical expression of MMP9 in a 56 year old female patient

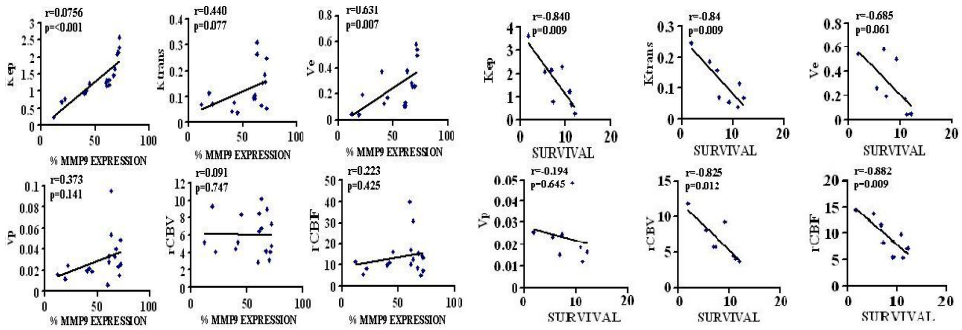

Fig 2: Graphs showing correlation of various DCE MRI metrics with percentage immunohistochemical expression of MMP9 and duration of survival (in months)

Discussion: In the present study we have found a significant positive correlation of Kep with MMP-9 expression, no matter which perfusion matrix was considered for its maximum value in its quantitation. MMP-9 expression has been shown to be associated with the disruption of blood brain barrier. In a recent study the expression of MMP-9 was proposed as marker of BBB disruption and disease activity in brain tuberculoma, and it correlated with Ktrans. The mechanism and extent of BBB disruption is different in infective lesions as compared to neoplastic lesions, which might have resulted in lack of correlation between MMP-9 and Ktrans among GBM patients. On the other hand a strong significant correlation between MMP-9 expression and Kep indicate that Kep is a more appropriate representative of MMP 9 expression in GBM as compared to other perfusion metrics. It has been reported that increased MMP-9 expression portends tumor progression.[7] The significant negative correlation of MMP-9 expression with the duration of survival of patients as observed in this study lends support to this hypothesis. We suggest that Kep appears to be a promising surrogate marker for MMP-9 expression in high grade gliomas and may be used in the management of patients with GBM.

References: 1-Persidsky Y et al. J Neuroimmune Pharmacol 2006;1:223-36, 2-Parks WC et al. Nat Rev Immunol 2004;4 :617-629, 3-Bart J et al. Cancer Treat Rev 2000;26 :449-62, 4-Lo EH et al. J Neurosci Res 2002;69:1-9, 5-Roberts HC AJNR 2000;21:891-99, 6- Singh et al. *J Magn Reson Imaging* 2007;26 :871-80, 7-Cerebrospinal Fluid Res 2008;5:1.