

R. K. Gupta¹, M. Haris¹, N. Husain², M. Husain³, K. N. Prasad⁴, M. Pauliah¹, C. Srivastava³, M. Tripathi⁵, M. Rastogi³, S. Bihari⁶, D. Rathore⁷, R. S. Rathore⁷

¹Department of Radiodiagnosis, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, ²Department of Pathology, King George's Medical University, Lucknow, Uttar Pradesh, India,

³Department of Neurosurgery, King George's Medical University, Lucknow, Uttar Pradesh, India, ⁴Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India,

⁵Department of Anaesthesiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, ⁶Department of Neurosurgery, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India,

⁷Department of Mathematics, Indian Institute of Technology, Kanpur, Uttar Pradesh, India

Introduction

Brain tuberculomas form a large percentage of intracranial mass lesions in the developing countries. It is possible, to recognize this entity more precisely on MRI¹. We have been able to separate cellular component from necrotic component of tuberculoma with the help of MT MRI². High expression of vascular endothelial growth factor (VEGF) has been shown in high-grade tumors as well as cerebral infarction; however it has also been demonstrated in the activated macrophages of lung as well as meningeal tuberculosis³. Perfusion MRI has been extensively used in grading of gliomas. In this study we performed dynamic contrast enhanced (DCE) MRI in 9 patients with brain tuberculomas with an aim to correlate relative cerebral blood volume (rCBV) and relative cerebral blood flow (rCBF) with immunohistochemical markers of angiogenesis like microvascular density (MVD), vascular endothelial growth factor (VEGF) and contrast enhanced MRI derived cellular and necrotic fractions of the lesion. In addition, we also followed 6 patients with specific chemotherapy at different time points to assess its impact in predicting the therapeutic response.

Material and Methods:

Subjects: We examined 15 patients [9 females (mean age: 18 years), 6 males (mean age: 14 years)] of tuberculomas with DCE MRI. Final diagnosis in all these cases was based on the characteristic imaging features and therapeutic response and histopathology in 9 patients.

Data acquisition: MR imaging was performed on a 1.5 tesla GE scanner using quadrature transmit-receive head coil. T2 [TR/TE/NEX=4900ms/85ms/2), T1 (TR/TE/NEX=650ms/9ms/1) and T1 weighted MT imaging (TR/TE/NEX=1200/14/1), off-resonance pulse with frequency offset-1200 KHz) were performed in the axial plane with 5 mm slice thickness, 256 x 256 matrix, 240 x 192 mm² field of view (FOV), with no slice gap. In vivo single voxel PMRS was done using point resolved spectroscopy (PRESS) with TE/TR/NEX=135/1500/8. A bolus of Gd-DTPA in the dose of 0.1 mmol/kg was injected at the 10th phase, intravenously with the help of pressure injector at the rate of 3.5 ml/sec, followed by with 40-mL saline flush. Using a three-dimensional spoiled gradient recalled echo (3D-SPGR) sequence (TR/TE-6.6/2, α -15°, FOV=360 x 270mm, slice thickness- 5mm, matrix size-256x128) brain images covering the lesion were obtained in the axial plane at 7-seconds interval for 32 phases. Post-contrast T1-weighted imaging was performed using the same parameters as was done for pre-contrast T1 weighted imaging. The same imaging protocol was repeated around every 4.5 months for a period of nine month on the patients who were put on conservative anti-tuberculous therapy.

The perfusion maps were generated by means of mathematical deconvolution of a tissue concentration curve with the arterial input function (AIF)⁴. Total ten ROIs were drawn on the region showed highest color-coded map on each slice depicting the cellular component of the lesion as well as on contralateral side, the ratio was taken for the statistical analysis. The ROIs were also placed over necrotic portion for the calculation of rCBV and rCBF. The total, necrotic and cellular fraction volumes of the lesion were calculated from the post-contrast T1 images using in house developed software.

Histopathology: Dewaxed and dehydrated 6µm sections of formalin fixed and paraffin embedded excised tuberculomas (n=9) were immuno-stained for polyclonal antibody against human VEGF (A-20) at a dilution range of 1:100, and monoclonal antibody to CD-34 antigen at a dilution of 1:50 using the standard protocol. All immunostained slides were digitized under 40X objective using Canon Power Shot G5 camera and the captured images were subjected to morphometry using the Biovis image analysis system. The MVD and percentage area of VEGF were calculated for ten high power field (HPF) in each case of excised tuberculoma.

Result: All the lesions appeared hypointense on T2, isointense with slightly hyperintense rim on T1 and showed hyperintense rim on MT T1 weighted images. CD34 highlighted the MVD in granulomatous area of all 9 excised tuberculoma. VEGF immunoreactivity was observed in the epithelioid cells as well the langhan's giant cells of the granulomas and was consistent in macrophages bordering the zone of necrosis. VEGF expression was also observed in a fair number of astrocytes. The rCBV of cellular portion correlated significantly with cellular fraction volume, MVD and VEGF in excised tuberculoma and did not show any correlation with necrotic and total volume fractions. The rCBF did not correlate significantly with any of the above parameters. There was also strong positive correlation between MVD and VEGF. The rCBV and rCBF obtained from the necrotic portion did not show any significant correlation with MVD and VEGF. In case of follow-up study the cellular volume of tuberculoma showed significant decrease over three time points along with significant decrease in rCBV. The necrotic volume did not decrease significantly over the three time points.

Discussion: Significant positive correlation among the rCBV, MVD and VEGF suggests that rCBV may be used as a measure of angiogenesis in tuberculomas and can be used to predict the response to anti-tuberculous therapy. Our findings confirm that a high angiogenic activity in the wall of the tuberculomas is due to the up-regulated expression of VEGF. This was further confirmed by a strong direct correlation between MVD and VEGF. Significant reduction in the cellular component in case of follow-up patients is probably due to the better penetration of the drugs through the increased vascularity seen in the cellular tuberculomas that may be extrapolated from the increased rCBV, increased MVD and up-regulated expression of VEGF that was shown in the excised tuberculomas. A decrease in the rCBV with treatment at different time points suggests decreased angiogenesis and decrease in the cellularity of the lesion. No significant change in necrotic fraction with treatment suggests that in a large tuberculoma with predominant necrotic fraction, it may be futile to give a trial of anti-tuberculous therapy as they may not respond to therapy and may require surgery.

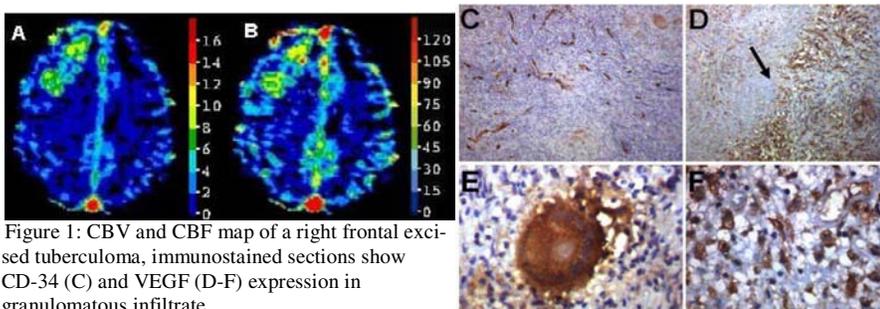


Figure 1: CBV and CBF map of a right frontal excised tuberculoma, immunostained sections show CD-34 (C) and VEGF (D-F) expression in granulomatous infiltrate.

TABLE-1 Correlation Table

Correlation	r value	P value
rCBV vs Cellular volume	0.908	0.001
rCBV vs MVD	0.949	<0.001
rCBV vs VEGF(%)	0.906	<0.001
MVD vs VEGF(%)	0.928	<0.001

References:

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- 4- Ostergard L et al. Magn Reson Imaging 1996; 36: 715-25.