Biological Correlates of Diffusivity in Brain Abscess

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Introduction: Low diffusivity on DWI is usually indicative of brain abscess, in patients with cystic intracranial mass lesions. However all the abscesses do not show low ADC and some of the cystic lesions like metastases have also been reported to have low diffusivity. It is possible to quantify necrotic fraction and cell density in brain tumors using DWI. A number of studies have described the possible causes of restricted diffusion in brain abscess and attribute it to a combination of inflammatory cells, necrotic debris, viscosity and macromolecules in the pus, however the exact contribution of these components to the restricted diffusion in brain abscess is not available in the literature. This study was designed to evaluate the role of various biological parameters like intact inflammatory cells, macromolecules and viscosity in contributing to the restricted diffusion in patients with brain abscess.

Methods: Patient study: 41 patients with brain abscess, included 35 males and 6 females with age ranging from 1-56 years (mean age 22 years). There were 36 patients with pyogenic abscesses and 5 with tuberculous abscesses. Final diagnosis was based on the isolation of the offending organism on culture (n=32). In remaining 9 patients the pus did not grow any microorganism and was termed as sterile. DW echo planar imaging (EPI) in axial plane using single shot EPI-SE pulse sequence with TR/ TE = 10.5 s/ 110 ms (minimum), field of view = 24×24 cm, nex = 2, slice thickness = 5 mm, no inter-slice gap with matrix size of 128×256 was performed. Diffusion sensitizing gradients were applied sequentially along the three orthogonal directions with diffusion sensitivity of b = 0 and 1000 s/mm^{2}with ramp sampling on. It has been shown that the ADC values correlate significantly with intact cell density in tumors in human as well as in experimental animals. We found brain abscesses with different pattern of low diffusivity: i) homogeneously low restriction ii) layered restriction iii) heterogeneously restricted (Fig 1 a-c). On the basis of inverse correlation published between cell density and ADC in animal tumor model and human glioma, we presumed that areas with bright signal on DWI were predominantly cellular in nature. The lesion was considered as bright if signal intensity on DWI over the lesion is higher as compared to the corresponding contralateral region. On this basis we found that region with ADC value $\leq 0.9\pm0.03\times10^{-3}$ mm^2/s are considered as cellular in this study. We calculated restricted and unrestricted lesion volumes based on cut off ADC value ($\leq 0.9\pm0.03\times10^{-3}$ mm^2/s) and subsequently volume and cell density over restricted area from the cell density per mm^3 of the pus obtained after aspiration/surgery was modeled. The restricted and unrestricted volumes of the abscess cavity were obtained by the software developed in-house using segmentation. Pus from these lesions was collected at the time of surgery and biological parameters namely viscosity, viable cell density and concentration of total extracellular proteins were measured within two hours of its aspiration. We identified non-viable cells by trypan blue (0.5%) staining and percent viability was calculated. Total Protein in supernatant of the pus was measured by Lowery method. Viscosity was measured using Ostwald's viscometer. In case of thick pus, we had used bench top viscometer.

Ex vivo study**:** The aspirate from the brain abscess (n=10) was taken in a 2 ml appendrof tube, inserted in to a 50 ml plastic tube filled with 2% agarose gel and DWI was performed before and after centrifugation using same imaging parameters as in case of patient study. Tube was centrifuged and level formed between the cellular/debris and fluid portion of the pus. All the biological parameters were again calculated using the above mentioned techniques. Cell density was determined in pellet after centrifugation. In order to further validate our results, ex-vivo imaging of the white blood cells isolated using lymphoprep lymphocyte isolation media and suspended these in the plasma. We necrosed the desired fraction of the total cell density (10-90%) by freezing at -70ºC and thawing of these cells multiple times to see the effect of necrotic and intact cells on ADC.

Results: On ex vivo imaging, ADC value of pellet ($0.46\pm 0.12\times10^{-3}$ mm^2/s) was significantly lower as compared to ADC of supernatant ($1.71\pm 0.21\times10^{-3}$ mm^2/s) as well as whole pus ADC ($0.97\pm0.14\times10^{-3}$mm^2/s) in 10 samples from brain abscess at 19ºC. Viable cell density was 225000 $\pm$ 211837 cells/mm^3 having more than 95% intact pus cells, viscosity of the pus was 53.5 $\pm$ 8.9 centipoise and extracellular protein concentration was 74.5 $\pm$ 20.5 mg/ml. Cell density of pellet inversely correlates with restricted ADC (r = -0.67, p= 0.04).Average ADC and ADC of restricted area of all the lesions were $0.927\pm 0.39\times10^{-3}$ mm^2/s and $0.646 \pm 0.08\times10^{-3}$mm^2/s respectively. Average ADC value of sterile abscesses was $1.25 \pm 0.68\times10^{-3}$ mm^2/s and was significantly higher (p= 0.004) than average ADC of culture positive lesions ($0.835 \pm 0.193\times10^{-3}$ mm^2/s). Viscosity of the pus from all brain abscesses was 60.76± 70.9 centipoise (range 1.6-321 centipoise), viable cell density with more than 95% intact pus cells was 147910 ± 140404 cell/mm^3 and total extracellular protein was 59.07 ± 25.36 mg/ml supernatant of the pus. Multivariate analysis showed a significant inverse correlation of the cell density with restricted ADC. Restricted ADC did not correlate significantly with protein concentration as well as viscosity. Linear regression fit analysis showed a highly significant (r = -0.88, P=0.0001) inverse correlation of cell density with restricted ADC (Fig. 1d). In ex-vivo white cell experiment, viscosity of the 10 samples of WBC did not much change with decrease in cell density at 23ºC. More than 98 % of the white cells were intact on 0.5 % trypan blue staining on initial experiment that reduced gradually to less than 10%. There was a significant inverse correlation (r = -0.95, p= 0.004) between ADC and cell density.

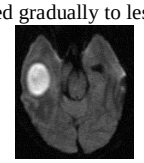
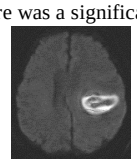
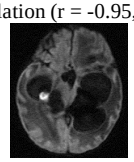
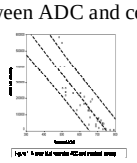

Fig. 1a Homogeneously restriction. Fig 1 b: layered restriction. Fig 1 c: heterogeneously restriction Fig 1 d: Linear fit.

Discussion: The significant inverse correlation between cell density of pus and restricted ADC from brain abscess suggests that intact cells in pus of brain abscess are mainly responsible for the restricted diffusion in brain abscess. Significantly high average ADC of sterile abscess than restricted ADC of culture positive abscesses is attributed mainly to the long-term use of antibiotics prior to the culture of the pus. These observations suggest that an increase in ADC in an abscess on in vivo following antibiotic therapy while managing the brain abscess conservatively may help in predicting response to therapy in these patients. We conclude that viable cell density in brain abscesses is the most important biological parameter responsible for restriction of diffusion on DWI.