Diffusion Tensor Fractional Anisotropy is a Potential Surrogate Marker for Neuroinflammatory Molecules in Brain Abscess

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Introduction: Brain abscess (BA) is a focal suppurative process within the brain parenchyma. Inverse correlation has been reported between intact inflammatory cell counts from BA cavity and corresponding low mean diffusivity (MD)[1] whereas increased diffusivity on treatment is considered as a marker for improvement.[2] High fractional anisotropy (FA) in the cavity of BA patients has been reported and are comparable to normal white matter.[3] High FA is usually interpreted as representing white matter tracts in the brain parenchyma. The contents of the BA cavity are intact inflammatory cells, necrotic cellular debris, and proteinaceous exudates.[1] Multifold enhancement, persistence and depletion of various neuroinflammatory molecules (NMs) with time has been reported in animal models of brain abscess.[4-5] Temporal increase in diffusivity on treatment/aspiration has been reported in patients with BA[2] and thus, it may be useful in monitoring the effect of antimicrobial therapy using noninvasive diffusion tensor imaging (DTI). Expression of intercellular cell adhesion molecule-1 (ICAM-1), a ligand on endothelial cell and lymphocyte function-associated antigen-1 (LFA-1), a receptor on all leukocytes is known to be associated with active inflammation. We have postulated that increased FA values inside the BA cavity may be attributed to the polarization and alignment of intact inflammatory cells secondary to cell adhesion molecules, which play an important role in cellular aggregation. We have prospectively analyzed FA values in the cavity as well as in the wall of the BA and quantitated neuroinflammatory molecules like ICAM-1, LFA-1, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) from the pus to quantify any relationship among these parameters. In addition we have shown the expression of adhesion molecules in the wall of the BA when it was available for histopathology.

Materials and Methods: Five patients with BA including 4 males aged from 1-50 years (median age 11 years) formed the study group. *Staphylococcus aureus* (n=3), *Sterptococcus uberis (*n=1), and *Nocardia species* (n=1) were isolated from pus culture of these patients. Conventional MR (magnetic resonance) imaging and DTI were acquired on a 1.5 Tesla MR scanner using standard quadrature birdcage head coil. DTI data were acquired using a single-shot echo planar dual spin-echo sequence with ramp sampling. The acquisition parameters were: TR=8sec/TE=100ms/number of slice=34-36/with contiguous 3 mm slice thickness/FOV=240mm/image matrix=256×256 (following zero-filling)/NEX=8/diffusion weighting b-factor=1000 s $mm^{-2.}$ The DTI data were processed as described elsewhere[6]. Size of the region-of-interests (ROIs) was guided by the lesion size and it was typically 2×2 pixels in the wall and 8×8 pixels in the cavity of BA with shape varying from elliptical to rectangular. Surgery was performed within 12 hours of MR study and pus was collected in liquid nitrogen at the time of surgery/aspiration in sterilized vial. Abscess wall following total excision of the BA was available in three patients for histopathological evaluation.

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) assay for NMs (ICAM-1, LFA-1, TNF-α and IL-1β): Total RNA was extracted from the pus using Qiagen RNeasy kit (Qiagen, Inc., CA, USA) according to manufacturer's instructions. Briefly, 1×10^6 cells were lysed and homogenized under highly denaturing conditions, cell lysates were then applied to an RNeasy spin column, and contaminants were washed away, followed by RNA elution with 50 µl (micro liter) of DEPC (diethylpyrocarbonate) treated water. RNA was quantified by spectrometry and equal amount of RNA were taken in each RT-PCR reaction mixture. 100 nanogram of RNA was reverse transcribed using MuV reverse transcriptase and oligo dT primers to make cDNA (complementary DNA). RT-PCR analysis of cDNA was conducted in 25 µl reaction mixture using Taq DNA polymerase and primers specific for human gene: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ICAM-1, LFA-1, TNF- α and IL-1β. The reaction was conducted for 35 cycles in MJ Research Cycler (Perkin Elmer, USA), the annealing temperature was 58ºC. PCR products were fractionated by 2% agarose gel electrophoresis and photographed under Ultra Violet illumination. Band intensities were quantified by densitometric scanning. To normalize mRNA levels, density of LFA-1, TNF-α, IL-1β and GAPDH bands from the same lane were scanned, and data were calculated as the ratios of band intensity values of LFA-1, TNF-α and IL-1β relative to band intensity of GAPDH and are presented as a density ratio.

Immunostaining of the Brain Abscess Wall: Immunostaining of 3/5-BA wall was performed according to manufacturer's instructions, using anti LFA-1, ICAM-1 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz: California, USA) of mouse origin recommended for the detection of LFA-1, ICAM-1 of human origin.

Results: Intact inflammatory cell counts of BA pus varied from 5000-220000 cells/ mm^3 (n=5). FA from the wall was significantly (p=0.04) high compared to FA of cavity whereas MD of wall was significantly (p = 0.0001) low compared to MD of cavity of BA (n=5). Positive correlations between FA and NMs and among NMs were found (Table). ICAM-1 was not detectable by RT-PCR. Immunostaining of ICAM-1 (red) and LFA-1 (green) antibodies were detected outlining cell surface, where colocalization of these two antibodies were visualized (Fig 1E) in the section from wall (n=3) of the BA (Fig 1B) from the patient whose MR images are given (Fig 1A-E).

Parameters (n=5)	Values (±SD), (n=5)	Pearson's correlations (r) & Significance (p) (n=5)
FA of BA wall	0.52 ± 0.04	r, p ; FA vs a (n=5) =0.48; 0.170
FA of BA cavity	0.25 ± 0.05	r, p ; FA vs b (n=5) =0.76; 0.010
MD of BA wall	0.46± 0.19	r, p ; FA vs c (n=5) =0.82; 0.003
MD of BA cavity	0.89± 0.38	r, p; a vs b (n=5) = 0.88; 0.003
IL-1β /GAPDH[a]	2.8 ± 1.53	r, p; a vs c (n=5) = 0.89; 0.001
LFA /GAPDH[b]	3.2 ± 1.91	r, p; b vs c (n=5) = 0.88; 0.003
TNF-α /GAPDH[c]	1.3 ± 1.03	

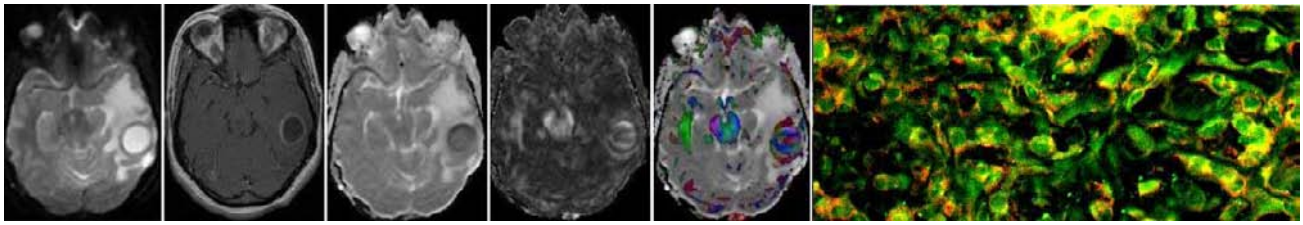

Fig. 1A. T2 **B.** Post contrast T1 **C.** MD **D**. FA **E.** Color-coded FA, **F.** Immunostaining of ICAM-1 (red) and LFA-1 (green) in abscess wall.

Discussion: Positive Pearson's correlations between FA with NMs and among NMs suggest that increase in FA is a noninvasive surrogate marker of active neuroinflammation in the cavity and the wall of BA. Interaction of ICAM-1 with LFA-1 in the wall of BA on immunostaining is suggestive of active neuroinflammation and significantly high FA (p = 0.04) on the wall of BA can be used as a noninvasive marker for adhesion molecules. NMs are reported to deplete with time in animal models[4-5] and temporal increase in diffusivity on treatment/aspiration in patients with BA[2] and thus, it may be useful in monitoring the effect of antimicrobial therapy using noninvasive DTI. Various NMs have been reported to increase in the human blood in response to inflammatory process[7] as well as in the experimental BA models[4-5]. TNF-α and IL-1 are known to induce ICAM-1 expression on human brain endothelial cells.[8] Leukocyte adhesion by involvement of ICAM-1 and LFA-1 has been proved and quantitated by an ex-vivo microkinetic study[9], supportive of above in vivo study. In conclusion, DTI derived FA measure can be used as surrogate marker of neuroinflammatory molecules and may be of value in assessment of activity of the disease non-invasively.

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