Explanation of High Fractional Anisotropy Value in the Wall and Cavity of the Brain Abscess Differs as Evident by Histology and Immunohistochemistry

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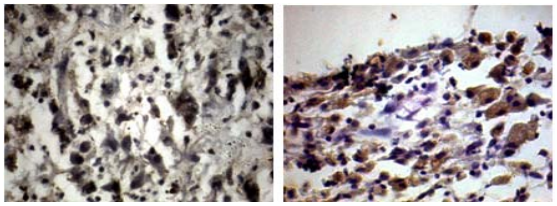
Introduction: Brain abscess (BA) develops in response to a parenchymal infection with pyogenic bacteria, beginning as a localized area of cerebritis and evolving into a suppurative lesion surrounded by a well-vascularized fibrotic capsule. The leading etiologic agents of BA are the *Streptococcus* species and *Staphylococcus aureus,* though a myriad of other organisms have also been implicated[1]. The role of proinflammatory molecules such as tumor necrosis factor-alpha (TNF-α) and interleukin1-beta (IL1-β) has been reported in the development of BA[2]. These proinflammatory molecules in turn induce the expression of various cell adhesion molecules including selections, intercellular cell adhesion molecules (ICAMs), vascular cell adhesion molecules (VCAMs). These cell adhesion molecules facilitate the extravasation of peripheral immune cells, perpetuating the anti-bacterial immune response that is thought to contribute, at least in part, to the development of BA[3]. Expression of 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. Gupta et al. reported for the first time high fractional anisotropy (FA) inside the BA cavity and postulated that this is due to the upregulation of various adhesion molecules on inflammatory cells, which confers the structured orientation of these cells in the cavity[4]. Since the wall is composed of concentric layers of collagen fibres intermixed with the neutrophils and macrophages that show the expression of ICAM-1 and LFA-1 that we have quantified therefore in the current study, we wanted to see the difference in the relationship between ICAM-1 and LFA-1 expression and FA in the BA wall and cavity and its possible explanation vis-à-vis histology.

Materials and Methods: *Subjects:* Eight patients with BA (5 males and 3 females; median age, 28 years; range, 1–50 years) were consecutively studied. Patients with an initial diagnosis of BA, based on conventional MRI, including diffusion imaging as well as *in vivo* proton MR spectroscopy, were selected. The diagnosis of BA was finally confirmed at surgery and by the results of the culture of the aspirated pus. *Staphylococcus aureus* (n=3), *Sterptococcus* sp. *(*n=3), and *Bacteroides* sp. (n=2) were isolated from pus culture of these patients. *Imaging protocol:* Conventional MRI and DTI data were acquired on a 1.5 Tesla GE MRI scanner using a quadrature birdcage head coil. DTI data were acquired by using a single-shot echo planar dual spin echo sequence with ramp sampling. The diffusion weighting b-factor was set to 1000 sec mm^{-2}. The other acquisition parameters were TR=8 sec, TE=100 msec, number of axial sections=34-38, slice thickness of 3 mm with no gap, field-of-view=240 mm^2, image matrix of 256 × 256 (following zero-filling) and NEX=8. To calculate the FA and mean diffusivity (MD) in the BA cavity and wall, we performed an automated segmentation by using the in-house developed JAVA based software. DTI-derived metrics (FA and MD) was calculated from the abscess wall by placing a total of 5 region-of-interest(s) (ROIs) (12-16mm^2) in the region showing highest FA value as depicted from the FA map, while in case of cavity, ROI was placed in the whole cavity for the quantitation. *Neuroinflammatory molecules* (*NMs) quantification from the pus:* RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) assay were performed to quantify NMs (LFA-1, TNF-α and IL-1β) from the BA cavity aspirate, using the method described in detail elsewhere[4]. Band intensities of these NMs were quantified by densitometric scanning. To normalize mRNA levels, density of LFA-1, TNF-α, IL-1β and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) bands from the same lane were scanned, and data were calculated as the ratios of band intensity values of LFA-1, TNF-α, IL-1β relative to band intensity of GAPDH and are presented as a density ratio. Human soluble ICAM-1 in pus was quantitatively measured by human soluble ICAM-1 enzyme-linked immunosorbent assay (ELISA) (Cat. No. BBE1B, R&D Systems, Minneapolis, Minn).
Immunostaining of the brain abscess wall: The 6-µm thin sections of BA wall were immunostained with polyclonal antibody against human ICAM-1 (Santa Cruz Biotechnology, USA) and monoclonal antibody against LFA-1 (Santa Cruz Biotechnology, USA) using standard protocols. Morphometric analysis using percentage area as a parameter for ICAM-1 and LFA-1 was done in digitalized images (Leica DFC 320 camera mounted on Ziess Axiolab microscope) using BIOVIS image analysis system (Expert Vision, Mumbai, India). The percentage of 5 areas with maximal positive staining for ICAM-1 and LFA-1 expression was calculated at 40X resolution.
Bivariate Pearson's correlation was performed to study the relationship between DTI metrics (FA and MD) and NMs.

Results: FA in the abscess wall was higher (0.35±0.08) compared to FA of cavity (0.31±0.07) whereas MD of wall was lower (0.68±0.20) compared to MD of BA cavity (0.72±0.31). Positive correlations between FA and NMs and among NMs quantified from aspirated pus were found. A significant positive correlation was observed between FA and ICAM-1 and LFA-1 expression and among these adhesions molecules in the abscess wall (Table). No significant correlation was observed between MD and NMs. Immunostaining of ICAM-1 and LFA-1 antibodies were visualized in the histiocytes of BA wall (Figure).

Parameters (n=8)	Values (±SD), (n=8)	Pearson's correlations (r) & Significance (p) (n=8)
[a]FA of BA wall	0.35±0.08	r, p ; [a]FA vs c =0.63, 0.000 r, p ; [a]FA vs d =0.50, 0.007 r, p ; [b]FA vs e =0.81, 0.015 r, p; [b]FA vs f =0.75, 0.031 r, p; [b]FA vs g =0.75, 0.032 r, p; [b]FA vs h =0.94, 0.000 r, p; c vs d = 0.54, 0.003 r, p; e vs f = 0.70, 0.051; e vs g = 0.81, 0.014; f vs g = 0.84, 0.008; e vs h = 0.91, 0.002; f vs h = 0.86, 0.005; g vs h = 0.9, 0.001
[b]FA of BA cavity	0.31±0.07	
MD of BA wall	0.68±0.20	
MD of BA cavity	0.72±0.31	
% of ICAM-1expression[c] of wall	6.42±1.54	
% of LFA-1expression[d] of wall	1.45±0.82	
IL1-β/GAPDH[e] of cavity	2.79±0.54	
LFA-1/GAPDH[f] of cavity	3.18±0.70	
TNF-α/GAPDH[g] of cavity	2.98±0.67	
ICAM-1[h] of cavity	125.27±36.83	



A **B**

Figure: Immunohistochemistry of the abscess wall. (A, B), ICAM-1 (A) and LFA-1 (B) expression visualized in the histiocytes of BA wall (Labeled Streptavidin Biotin Horseradish Peroxidase System×525×digital magnification).

Discussion: In this study, FA positively correlated with the NMs quantified from aspirated pus as well as ICAM-1 and LFA-1 expression in the BA wall. Although the correlation was significant in both cavity and wall, however the cavity showed a better correlation coefficient compared to wall. This shows the maximum expression of various adhesion molecules on the inflammatory cells mainly in the neutrophils and macrophages. The cavity contains only the inflammatory cells which aggregate due to the upregulation of adhesion molecules however; the wall has a combination of concentric layers of collagen fibres intermixed with the neutrophils and macrophages. In the wall, the expression of ICAM-1 and LFA-1 was seen mainly in the macrophages and not in the collagen fibers. FA values in the wall was observed to be higher compared to the cavity even when ICAM-1 and LFA-1 were not expressed in the collagen fibers and hence showed lower correlation coefficient than the cavity. This suggests that the concentric layers of collagen fibers which provide structural orientation are responsible for higher FA in the wall compared to the cavity. It has been also shown that the laminar structure of collagen fibers in the heart is responsible for the increased FA[5]. We conclude that in the BA, there may be different explanations for increased FA in the cavity and wall substantiated by histology and immunohistochemistry.

References: 1.Townsend GC, et al. Adv Internal Med 1998;43:403–47, 2. Kielian T. J Neuroinflammation 2004;17:1–16, 3. Kielian T. Front Biosci 2004;9:732–50, 4. Gupta RK, et al. AJNR 2008;29:326-332, 5. Helm PA, et al. Magn Reson Med 2005;54:850-859.