Correlation of fractional anisotropy (FA) changes in demyelination lesion with its surrounding edema in an experimental model

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INTRODUCTION: Multiple sclerosis (MS) is a chronic demyelinating disease of the CNS characterized by the loss of myelin due to immune mediated inflammation leading to defective signal conduction along the CNS. The pathological features observed in MS are similar to that found in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Diffusion MRI allows quantitative estimation of demyelination and enables better understanding of tissue characteristics due to its sensitivity to detect the translational motion of water molecules. A detailed investigation needs to be carried out to evaluate the pathophysiology of the disease at various stages which is difficult in humans due to difficulty in identifying the exact location and onset of the disease. Thus, application of DTI to animal models of demyelination enables better insight of the mechanism underlying the disease development and its pathophysiology.

METHODOLOGY: Demyelination was induced by stereotaxic injection of 0.1 µl of 1% LPC in the internal capsule area of the rat brain (n =30, male Wistar rats; body weight = 150 to 250 gms). MR experiments were carried out at 4.7 T (BIOSPEC Bruker, Germany) using a 72 mm resonator as transmit/receive coil. The rats were sequentially monitored using diffusion studies at various time periods of demyelination [pre-acute (days 3-5), sub acute (days 5-7), acute (days 9-12) stages] and remyelination on days 15 and 20. DTI experiments were carried out using a EPI sequence on the slices seen on T2-W images using the following parameters: TR/TE = 3000/38.3 ms, 30 diffusion directions and three 'b' values = 0, 250, and 855.27 s/mm^2 Due to mortality, not all rats could be monitored at all time points. Uniform circular ROIs of 4 pixels (area = 0.0039cm^2) were selected from the lesion area as well as from the edematous area surrounding the lesion for calculation of fractional anisotropy (FA). For statistical analysis Student t-test was used to compare means of FA in lesion and edema. Pearson correlation was used to correlate the FA values between lesion and edema. The institute animal ethics committee approved the study.

RESULTS: Out of the 30 LPC injected rats, demyelination was observed in 18 rats as hyperintense region in the internal capsule (ic) area on T2 - weighted images. Of these 18 rats, FA could be analyzed sequentially only in 17 rats. In 14/17 rats, edema was observed around the lesion that also appeared hyperintense on T2-W images but with higher intensity. The lesion and the surrounding edema appeared hypointense on an FA map. The FA of lesion and edema decreased during demyelination from day 3 (n = 13, FA_{lesion} = 0.27±0.03; n = 9, FA_{edema} = 0.21± 0.02) till day 11 (n = 6 FA_{lesion} = 0.17 ± 0.03; n =4, FA_{edema} = 0.14 ± 0.02). Subsequently, the values increased from day 15 (n = 3, FA_{lesion} = 0.34 ± 0.03; n= 2, FA_{edema} = 0.20 ± 0.03) till day 20 (n= 2, FA_{lesion} = 0.47 ± 0.02; n = 2, FA_{edema} = 0.26 ± 0.03) indicating remyelination (Fig. 1). The FA_{edema} was lower compared to the lesion throughout the progress of de- and re-myelination and was statistically significantly lower on day 7 (p =0.007). At day 20, (during remyelination) the difference between FA_{lesion} and FA_{edema} was significant (p = 0.05). When FA_{lesion} and FA_{edema} were compared with the unaffected contralateral region, it was observed that FA_{lesion} was significantly lower during demyelination (i.e. upto day 11) while there was no significant difference between the two regions (lesion and contra-lateral area) during remyelination. However, FA_{edema} values showed significantly lower values on all days compared to the contralateral ic region. A Pearson correlation plot between FA_{lesion} and FA_{edema} revealed a positive correlation between the two with a correlation coefficient of 0.93 (Fig 2).

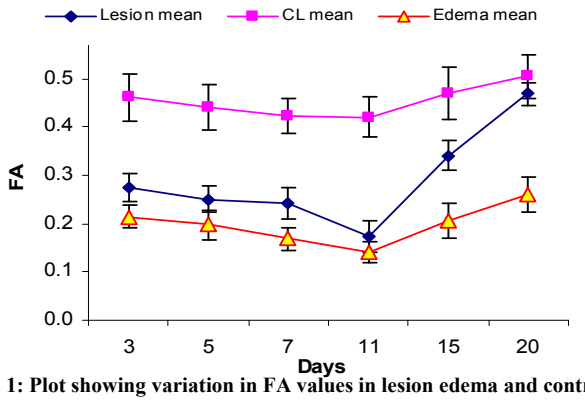

Fig. 1: Plot showing variation in FA values in lesion edema and contra-lateral region during the various stages of de- and re-myelination

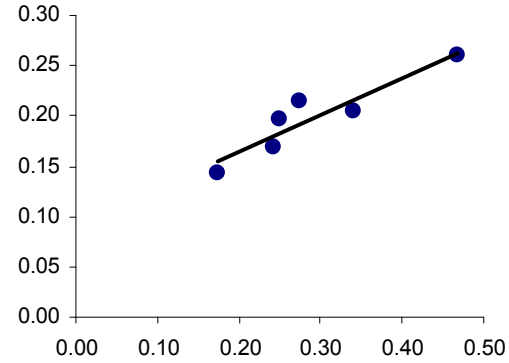

Fig. 2: Correlation Plot between FA_{lesion} and FA_{edema}

DISCUSSION: In many brain disorders such as ischemia, multiple sclerosis, EAE, epilepsy etc the cerebral endothelium gets affected. As a result, the blood-brain barrier (BBB) gets damaged or altered leading to a large increase in vascular permeability which consequently leads to vasogenic edema in the brain [1]. Studies in EAE have shown significant increase in BBB permeability and damage at the site of the lesion [2]. In the present study, we have calculated the FA values of the edematous region surrounding the lesion during the entire process of de- and re-myelination and compared it with the lesion in order to understand the mechanism underlying demyelination and edema formation. It was observed that as FA_{lesion} decreased during the progression of demyelination, FA_{edema} also decreased and it subsequently increased with FA_{lesion} during remyelination i.e. from day 15. This was observed as a positive correlation (0. 93) between the two on a Pearson correlation plot.

The decrease in FA of edema around the lesion may be attributed to the breakdown of the blood brain barrier which leads to interstitial and vasogenic edema around the demyelinating lesion [3]. As the lesion progresses, there is an increase in the extracellular free water mobility in the edematous area around the lesion due to widening of the extracellular space [3, 4]. Consequently, there is an increase in the diffusion in this region which tends to be isotropic. This was observed as decreased FA_{edema} during demyelination. The decrease in FA_{lesion} during demyelination is attributed to the damage to the myelin sheath around the nerve fibers [5]. On day 11, the lesion and surrounding edema were not distinguishable (p= 0.08). However, they were distinguishable based on their ADC values since the ADC of edema was significantly higher than the lesion [4]. Although ADC allows the differentiation between edema and lesion based on their diffusion rate, the directionality of motion cannot be monitored. DTI provides quantitative estimation of water mobility in tissues as a function of the direction of diffusion by the application of diffusion–sensitization gradients in multiple directions [6]. The FA obtained from the diffusion tensor measures anisotropy in the tissues. The lower FA value of edema compared to lesion could be explained by the fact that as demyelination occurs, the damage to the blood brain barrier is pronounced than the damage to the myelin sheath which occurs in a progressive manner till day 11. On day 11, maximum demyelination occurs and the diffusion of water molecules in the lesion tends to be isotropic [5]. During this time, the associated edema is also maximum and is isotropic. Hence, lesion and edema become indistinguishable during this stage on an FA map. As remyelination sets in, the repair of BBB also begins which leads to reduction in edema and this is observed as increased FA of lesion and edema from day 15. Vasogenic edema may resolve as the BBB gets repaired or ceases to leak as a result of regeneration of endothelial cells or due to repair of tight junctions. However, the recovery of the BBB is slower compared to the remyelination process and this is evident as significantly lower FA_{edema} compared to FA_{lesion} on day 20. Thus, DTI aid in understanding the pathophysiology of myelination, more so in distinguishing from associated edema during remyelination.

References:

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