A Refined DTI Study Shows Increased Sensitivity on an mTBI Study: Results Highly Correlated with Proteomic Findings

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Introduction: As an increasing public health problem, mild Traumatic Brain Injury (mTBI) affects approximate 1.2 million Americans annually [1]. Under-diagnosis with clinical-based symptoms and insensitivity of conventional MRI and CT to mTBI make its fast and early diagnosis a big challenge. Recent DTI studies on mTBI show that it is possible to detect the localized diffusive changes suggestive of axonal injury with either region-of-interest (ROI)-based methods [2, 3], or voxel-base morphometry (VBM) [4] study. Both methods have pros and cons: ROI-based analysis has strong statistical power, but its effectiveness largely depends on the hypothesis about specific injured regions, and it is very time consuming. VBM-based method makes no assumptions about locations of abnormality and is time efficient, but it suffers from lower sensitivity due to errors in co-registration and image smoothing. In this study, we investigated a refined mTBI-DTI analysis combining both the methods, utilizing ROI analysis on predefined mTBI vulnerable region in normalized space and exploring other regions with VBM. Quantitative evaluations were performed, and the DTI results were correlated with a blood biomarker proteomic analysis.

Methods: Six neurologically normal subjects with isolated mTBI and six age- and gender- matched controls were studied. For all patients, the initial Glasgow Coma Scale (GCS) was 13-15, and no intracranial injuries were found from CT scans. All images were obtained within 72 hrs of injury on a Siemens 3T Trio system with an 8-channel head coil. DTI sequence parameters were: TR/TE=10100/100ms, isotropic 2x2x2 mm voxel, matrix=128x128, iPAT (GRAPPA) acceleration factor=2, 60 diffusion gradient directions with b=700 s/mm^2 and one average, b=0 images with 10 averages. High resolution (1x1x1mm) 3D SPGR T_1W image and double GRE images were acquired for spatial normalization and susceptibility corrections. *Blood samples and proteomic analysis:* For each subject, serum samples were collected for proteomic analysis (within 6 hrs of impacts), with SELDI protein chip to analyze the anion exchange-fractionated serum. *Image Processing:* Home-built software was used for post-processing. Before DTI parameter calculation, additional eddy-current and field map correction steps were performed. *VBM analysis:* For each subject, DTI was first registered with T_1W images, which was in turn registered with MNI T_1 template. In order to avoid bias from mis-registration, an additional mask was generated for SPM statistics: A CV (standard deviation/mean) map based on spatial normalized T_1W images of all subjects was generated. Next, a mask image (Fig1, A) was generated by setting CV<10% with the assumed variation of intensity of T_1W image (normal appearance in T_1W for mTBI) within this range. With the assumption that there are common regions vulnerable to impacts in mTBI and there are also case- and subject- dependent damaged regions, two t-tests were performed: two-sample t-test for FA and mean <D> comparing the patient group to the normal group, and t-test comparing each individual patient to the normal group. Only hot spots survived the restricted FWE correction ($p<0.05$) were considered. *Comparison of two ROI-selection routines:* Six different ROIs: anterior and posterior Corpus Callosum (ACC, PCC), anterior and posterior limb of Internal Capsule (AIC, PIC), and External Capsule (EC) and Cingulum (CIN) were selected. The first five ROIs have been hypothesized to be vulnerable in mTBI [2]. In addition to drawing ROIs in each individual's brain, ROIs were also drawn once in the normalized T_1 template and then mapped back into individual subject's brain. For quantification, all ROIs were drawn four times by two ROI-drawing routines (within 4-day period) performed by the same person. The between-routine similarity and within-routine reproducibility were evaluated by the following criterions. For similarity, (1) True White Matter Coverage Ratio (TWMCR) as defined in Eq (1) was used to find overlapping of the ROI defined in the normalized space to the WM mask in the native space. (2) Common Ratio (CR): overlap ratio of generated ROI by two drawing routines on the same target tissue. (3) Paired t-test on equivalent of DTI measures at the same ROI generated by the two ROI routines. For each ROI of each ROI-drawing routine, there are 4 measurements for each subject. For each ROI region, paired t-test performed on 12 pairs of mean DTI measures, e.g. FA and <D>, of 4 measurements of each patient. $\alpha = 0.01$ was set to correct the multiple comparison issue. For repeatability, the coefficient of variance CV among four measurements, meanwhile, will serve as the quantitative measures.

Results: VBM analysis did not show significant difference in FA and <D> at the group level. Meanwhile, individual subject vs. control pool comparisons show four out of six patients have significant increase of <D> in different isolated spots (two in frontal lobe, one in temporal lobe and one in right corpus callosum). Further analysis with spherical ROIs placed at the center of these regions (with 4 mm radius) in the native space verified the results from the VBM analysis. No significant FA and <D> changes were found by ROI analysis at group level comparison between patients and controls. In two patients no significant changes were found by either VBM or ROI analysis. In five out of the six ROIs studied, there were no difference in measured FA and <D> between the two drawing routines ($p<0.01$ with multiple comparison correction). Only the FA in the cingulum was different in the two routines. The effectiveness of ROI-drawing in normalized space was also illustrated by the overall high TWMCR and CR ratios (Table 1) among the investigated ROI regions. Reproducibility of the two ROI-drawing routines with CV among four different measurements is also illustrated in Table 1 (median values of CV were listed). With most ROIs investigated, reproducibility of ROI-drawing in normalized space is better than in the native space, and it is higher for <D> than FA. For the ROI-based analysis, both ROI-drawing routines detected significant reduction of FA value at anterior corpus callusom in four out of six patients, which agree with the previous results [2, 3] from ROI-based analysis on mTBI. In the blood serum sample tests, seven over-expressed and one under-expressed mass-to-charge (M/Z) peaks were found in patients with detected DTI changes. In one of these peaks (4.3kDa), the peak intensity values among those with changed DTI measures were higher than among those without detected DTI changes ($p=0.032$) and among controls ($p=0.018$) (Fig2). Detection of abnormal expressions of certain proteins released by brain tissue in blood serum will potentially provide more insight of the pathology associated with mTBI.

Discussions: Our pilot study suggests that combined VBM and ROI-based analyses increase the overall sensitivity of DTI for early detection and diagnosis of mTBI. Quantitative analysis between ROI drawing in the normalized and native spaces shows the equivalence and repeatability of the two routines. Better repeatability of the proposed ROI-drawing routine will be beneficial for longitudinal study. Further evaluation of the method is still needed for more white matter structures with different background contrast. Mismatch of two ROI routines in cingulum region is partly due to the poor contrast of cingulum at T_1 template used for ROI-drawing in the normalized space. Normalized DTI measure maps, like FA-encoded color map, will aid in ROI drawing. For smaller structures like fornix, ROI-drawing in native space will still be the only choice. Further study on inter-person repeatability of two ROI-drawing routines is also needed. High correlation between DTI findings with proteomic results not only supports values of DTI on mTBI clinical studies, but also expands the investigation tool for study of the pathology associated with mTBI.

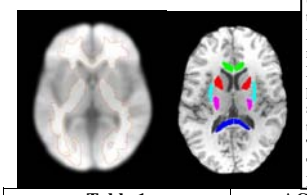

Figure 1 (left): A. custom-built mask to minimize the bias of mis-registration on SPM stats (overlaid on T_1 template). B. ROIs investigated: AIC -- Red, PIC -- Magenta, ACC-- Green, PCC– Blue, EC– Cyan. (CIN is not showed at this slice location).

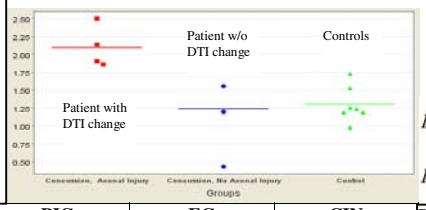

Figure 2 (right): Proteomic analysis shows that 4.3kDa peak in mTBI patient with detected DTI changes (**Red** squares) is significantly higher than normal (**Green** triangles) and non-DTI change patients (**Blue** circles). Two samples from each subject were analysis and plotted.

Table 1 (bottom): Quantitative comparison between two ROI-drawing routines.

$$Eq1: TWMCR = \frac{(ROI \cap WM\_MASK)}{ROI};$$

$$Eq2: CR = \frac{ROI\_SN \cap ROI\_NATIVE}{ROI\_SN \cup ROI\_NATIVE};$$

Table 1	ACC	PCC	AIC	PIC	EC	CIN
CV_ROI_SN(FA/<D>)	0.018/0.007	0.013/0.004	0.019/0.097	0.009/0.005	0.011/0.010	0.018/0.008
CV_ROI_NATIVE(FA/<D>)	0.019/0.015	0.018/0.017	0.032/0.093	0.012/0.006	0.017/0.005	0.019/0.006
TWMCR(median)	0.92(0.77/0.99)	0.90(0.77/0.95)	0.85(0.79/0.91)	0.95(0.79/0.90)	0.87(0.78/0.95)	0.60(0.48/0.69)
CR(median)	0.48(0.34/0.56)	0.43(0.2/0.57)	0.45(0.38/0.51)	0.40(0.35/0.48)	0.39(0.34/0.45)	0.20(0.14/0.20)

References:

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