Diffusion Tensor Imaging Parameters in Mild Traumatic Brain Injury and Its Correlation with Early Neuropsychological Impairment: A Longitudinal Study

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Objective: We explored the prognostic value of diffusion tensor imaging (DTI) parameters of selected white matter (WM) tracts in predicting neuropsychological outcome both at baseline and 6 months later among well-characterized patients diagnosed with mild traumatic brain injury (mTBI). Methods: Sixty-one patients with mTBI (mean age= 27.08, SD 8.55) underwent scanning at an average of 10 hours (SD 4.26) post trauma along with assessment of their neuropsychological performance at an average of 4.35 hours (SD 7.08) upon full Glasgow Coma Scale recovery. Results were then compared to 19 healthy control participants (mean age= 29.05, SD 5.84) both in the acute stage and 6 months post trauma. Results: The DTI and neuropsychological measures between acute and chronic phases were compared and significant differences emerged. Specifically, chronic phase FA and RD values showed significant group differences in the corona radiata, anterior limb of internal capsule, cingulum, superior longitudinal fasciculus, optic radiation, and genu of corpus callosum. Findings also demonstrated associations between DTI indices and neuropsychological outcome across two time points. Conclusion: Our results provide new evidence for the use of DTI as an imaging biomarker and indicator of white matter damage occurring in the context of mTBI, and they underscore the dynamic nature of brain injury and possible biologic basis of chronic neurocognitive alterations.

Key words: DTI, mTBI, neuropsychology, imaging biomarker, TBSS, ROI
INTRODUCTION

Mild traumatic brain injury (mTBI) constitutes approximately 75 to 85% of all head trauma cases.\textsuperscript{1} The long term outcome of mTBI, however, is not well characterized due to its considerable heterogeneity. One difficulty in accurately diagnosing mild neurotrauma relates to the frequent lack of radiological evidence to support the diagnosis, which often leads clinicians to diagnose mTBI based on clinical or cognitive symptoms known to overlap with other clinical conditions (e.g. hypoglycemic or vasovagal attacks and certain subtypes of mood disorders).\textsuperscript{2} Among the neuropsychological alterations that are commonly reported in patients with mTBI include impairment in attention, memory, psychomotor speed, and executive functions.

Mild neurotrauma is associated with traumatic axonal injury (TAI) which is described as a progressive event gradually evolving from focal axonal alteration to delayed axonal disconnection.\textsuperscript{3} Importantly, TAI is thought to represent one of the more common injuries observed in the aftermath of mTBI\textsuperscript{2}. These subtle alterations of brain tracts or fiber pathways have been visualized using diffusion tensor imaging (DTI), which enables better visualization of the extent of early microstructural changes post-mTBI.\textsuperscript{2,4-6} A variety of metrics can be generated through DTI scans, including fractional anisotrophy (FA) which is a per-voxel indication of the directionality of the underlying water diffusion. FA values range from 0 to 1, where FA = 0 would indicate non-directional diffusion (completely isotropic) and FA =1 would indicate a single direction of diffusion where the water molecules are restricted to diffusion only along a single axis (completely anisotropic).\textsuperscript{2,7} Reduced FA in the white matter is believed to reflect a loss of integrity, indicating possible damage to myelin or the axon membrane, reduced axonal packing, and/or decreased axonal coherence.\textsuperscript{2,8} Mean diffusivity (MD), on the other hand, describes the per-voxel average magnitude of water diffusion, regardless of diffusion direction. Differences in MD are thought to reflect overall
restrictions to the movement of water diffusion, examples being the variations within the intra and extracellular space. Radial diffusivity (RD) is defined as the diffusion of water perpendicular to white matter fibers which increases in response to demyelination and dysmyelination. The changes in axonal diameter or density can also influence the changes seen in RD. FA and MD values are usually inversely correlated with each other, as the myelination which enforces directionality (thus increasing FA), also represents a restriction to overall movement (thus lowering MD). The demyelinating changes as evinced by the changing RD, however, are not expected to occur in the first week post mTBI, despite the presence of axonal swelling and synaptic disruption.

In mTBI, widespread changes in FA are frequently observed, especially in the frontal, midline, and temporal regions. Studies have shown that these changes can be detected as early as a few days to weeks after neurotrauma, as well as months or even a year after the initial insult. The shearing forces of trauma can breach the vascular permeability of vessels (hence rupturing them), sever fibers and lyse cells which deregulates the normal homeostasis of the blood-brain barrier (BBB), and usually manifests as cerebral vasogenic and cytotoxic edema. Evaluation of DTI indices enables the differentiation of these edemas, which is crucial to predicting long-term neurological outcomes including neuropsychological performance (NP). Vasogenic edema commonly seen in mTBI is characterized by reduced FA, increased MD and RD, and is considered reversible. In contrast, cytotoxic edema characterized by an increased FA, reduced MD and RD, and is considered irreversible and therefore confers a poor prognosis.

To date, there are few studies that have been conducted longitudinally with acute mTBI samples to help elucidate the evolution of these DTI-based changes in mTBI over time. Unfortunately, existing studies have yielded generally equivocal findings. For example, some studies have reported decreased integrity of several tracts at different time
intervals (acute and chronic)\(^{30-35}\), although others report elevated FA and reduced MD in the acute stage.\(^{16,21,36}\) Since these findings may be inconsistent due to various methodological differences including patient recruitment, imaging protocol differences, varying intervals studied, sample size differences, and heterogeneity of injury severities, we aimed to clarify these longitudinal DTI changes using a whole brain white matter measurement strategy with Tract Based Spatial Statistics (TBSS)\(^{37}\) in mTBI and control groups. From the TBSS white matter skeleton of comparison across the subjects, we identified significant tract changes and correlated these regions with neuropsychological performances both at admission and 6 months post-injury in patients with mTBI. We also examined the relationship between anatomical correlates of the tracts and cognition in an effort to improve the prognostic values of DTI parameters in mTBI care.

**METHODS**

**Participants**

Sixty-one patients with mild head injury who presented to the emergency department of University of Malaya Medical Center, Kuala Lumpur for a consecutive 11-month period between April 1, 2013 and March 1, 2014 were prospectively recruited for this study. Patients were selected based on the inclusion and exclusion criteria as presented in Figure 1. For the purposes of this study, mTBI was defined as acute head injury, consisting of non-penetrating head trauma resulting in one or more of the following: confusion/disorientation; loss of consciousness (LOC) less than 30 minutes; posttraumatic amnesia (PTA - less than 24 hours in duration); and/or transient focal neurological signs or seizures; and Glasgow Coma Scale of 13 to 15 upon acute clinical evaluation. The flow of the study is presented in Figure 2. Nineteen healthy age-matched control participants were also recruited for this study.
All subjects meeting criteria for the study underwent computed tomography (CT) scans of the brain in the emergency department using a Siemens Somatom Sensation 16 CT scanner (Siemens AG, Berlin, Germany). Cross-sectional images of the brain were obtained craniocaudally from the base of skull to vertex. The scan parameters used were kVp 120, mAs 300, collimation of 16 x 0.75mm with standard brain and bone windowing. A neuroradiologist (NR) and a neurosurgeon (VN) who were blinded to the clinical diagnosis independently evaluated the images for each patient, and only patients who were deemed not requiring surgical intervention were included in this study. All subjects gave informed consent as required by the institutional research ethics committee and the hospital ethics committee (UM/EC Ref: 947.15)

**Study protocols**

Magnetic resonance imaging (MRI) and neuropsychological assessments were performed at admission and repeated again at six months post trauma (Figure 2). The healthy control participants were subjected to the same protocols as the patients upon admission (i.e. MRI and neuropsychological assessment).

**MRI data acquisition**

All consented subjects were imaged on a 3T MRI scanner (Signa HDx, General Electric, USA) using an 8 channel head coil. The imaging protocol included: (a) axial T1-weighted 3D fast spoiled gradient echo (FSPGR), TR = minimum 6.7 ms, TE = minimum 1.9 ms, FOV = 31 mm, matrix = 256 x 256, slice thickness = 1.2 mm, slice overlap =0.6 mm with image scan time of 3 min 48 s, (b) axial T2 weighted fast spin echo (FSE), TR = 4240ms, TE = 102ms, FOV = 24mm, matrix = 512 x 384, thickness = 5mm, spacing = 1.5mm with image scan time of 2mins 30s, (c) coronal gradient echo (GRE), TR = 655 ms, TE = 20ms, flip angle 15°, bandwidth 31.25, FOV = 24 cm, matrix = 320 x 256, thickness =...
5.0mm and spacing = 1.5mm with image scan time of 2mins 7s. The DTI sequence was obtained using these parameters: TR = 13 000 ms, TE = 81.2 ms, FOV = 24 mm, matrix = 128 x 128, slice thickness = 3.0 mm, 32 directions, diffusion-weighted factor, b = 700 s/mm$^2$ with image scan time of 7 min 22 s.

MRI Analysis

Tract Based Spatial Statistics (TBSS)

Voxelwise statistical analysis of the diffusion-weighted data was carried out using TBSS,\textsuperscript{37} part of the FSL software package (v5.0.6 University of Oxford, UK). Initial preprocessing involved corrections for head movement and eddy currents, brain tissue extraction, and fitting of the diffusion tensor model. These were carried out using the FSL eddy_correct, bet, and dtifit tools respectively. The standard TBSS analysis workflow was followed (V 1.2; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS/UserGuide), with the following specific options: nonlinear registration to the FMRIB58_FA standard-space image, generation of a study-specific mean FA image for skeletonisation, and a 0.2 threshold for the mean FA skeleton.

The voxelwise statistical analysis was carried out using the FSL randomise tool. A two-group unpaired $t$-test design was used for comparing the admission scans of the control and mTBI subjects. A paired $t$-test design was used for comparing the admission and follow-up scans of the mTBI subjects. These statistical analyses were carried out separately for FA, MD, and radial diffusivity (RD) values. In all cases cluster-based thresholding was used, and 0.05 was adopted as the threshold for significant clusters.

Region of Interest (ROI)

In addition, we obtained mean FA, MD and RD for all the tracts identified on TBSS
using region of interest analysis. The image-processing pipeline consisted of preprocessing, image registration, and analysis, utilizing the FSL (v5.0.6 University of Oxford, UK) and AFNI (v2011_12_21_1014 National Institute of Mental Health, USA) software packages. Initial preprocessing involved corrections for head movement and eddy currents, brain tissue extraction, and fitting of the diffusion tensor model. These were carried out using the FSL eddy_correct, bet, and dtifit tools respectively. For image registration, the FSL tool fnirt was used to carry out non-linear spatial registration of each subject to the FMRIB58_FA standard-space image, using the built-in FA_2_FMRIB58_1mm config file. This was assumed to be spatially compatible with the International Consortium of Brain Mapping (ICBM) DTI-81 atlas. Post-registration, composite axial slice images of the underlying FA and ICBM atlas tract outlines were generated at z=42 and z=82 for each subject. An experienced neuroradiologist (NR) verified the overall registration quality of these images. Finally, the AFNI 3dROIstats tool was used to map the predefined regions of interest (ROIs) to each individual subject, and to calculate the median fractional anisotropy (FA), mean diffusivity (MD), and radial diffusivity (RD) values for each tract.

**Neuropsychological Assessment**

All subjects underwent cognitive assessment using the Screening Module of the Neuropsychological Assessment Battery (S-NAB Form 1), which was performed by the neuropsychologist once the patient had recovered to a GCS score of 15, which occurred with an average turnaround time of 4.35 hours (SD.7.08) between time of trauma and full GCS recovery. The S-NAB comprises a comprehensive set of neuropsychological tests (refer to Table 1), with demographically corrected norms for adults between the ages of 18 to 97 years, assessing orientation and five cognitive domains (i.e., attention, memory, language, visuospatial and executive functions). This battery consists of 12 individual tests across the
five domains aforementioned. From these 12 tests, a total of 16 T scores are derived, 14 of which contribute toward five separate Screening Index (domain-specific) scores and one Total Screening Index score. The same subtests were repeated at 6 months by the same neuropsychologist using the S-NAB Form 2 in order to minimize practice effects.

Data Analysis

An independent samples t-test was used to establish whether the mean values of the FA, MD and RD of the selected WM tracts were significantly different between the healthy control and mTBI groups during the acute phase (or baseline exam). The same test was also used to investigate if the patients (at admission) performed differently from healthy control participants on the neuropsychological assessment. A paired t-test was used thereafter to ascertain how the WM tracts had changed over time. TBSS skeletonised image of the significant changes observed over time were processed to better visualize any significant changes. Spearman’s rho correlation coefficient was used to examine the association between white matter regions of interest (9 selected tracts) and neuropsychological performance (NP) over the different phases. Lastly, a simple frequency analysis of neuropsychological performance at 6 months post trauma was performed to determine the types of changes observed in neuropsychological status longitudinally (improved, unchanged or worsened).

RESULTS

Demographic and Clinical Characteristics

The demographic characteristics of the study patients and the healthy controls are presented in Table 2. The TBI group ranged in age between 18 and 53 years (mean: 27.08, SD: 8.55), was predominantly male (88.5%), and had a mean age of 29.05 (SD 5.84). There was no significant difference in the mean age of the healthy controls versus the TBI patient group (t (78)= .937, p= .256). However, the mean years of education differed significantly
between the groups [TBI: 11.52 (SD 1.94) versus controls: 15.95 (SD 2.01) years]. The admission GCS scores of the patients were within the range of 13 to 15 (M = 14.44, SD 0.74). Seventy-seven percent of the patients had experienced transient LOC while 73.8% reported PTA. The average time to scan of the patients during the acute phase was 10.01 hours (SD 4.26), while the chronic phase scan was performed at 6 months post-injury (mean = 6.05 months, SD 0.12). Out of the 61 patients, 33 underwent the chronic phase repeat scans [three patients were later dropped during the post scan processing as their scans had major artifacts in the Echo Planar Imaging (EPI)]. All healthy controls had no significant neurological findings.

Diffusion Metrics, Intergroup Differences and Intragroup Changes Over Time

Table 3 presents the mean FA, MD and RD values of both patients and healthy control participants during the acute phase. At baseline, the mTBI group showed significantly lower Splenium FA [t (78) = 2.196, p =0.04] when compared to the control group, while the MDa value was significantly higher in the mTBI versus control group in the posterior limb of the internal capsule (PLIC) [t (78) = -2.179, p= 0.03]; cingulum (CG) [t (78) = -2.29, p= 0.02]; optic radiation (OR) [t (78) = -3.176, p= 0.002] and splenium (SCC) [t (78) = -2.514, p= 0.02]. Group differences for the RDa values were seen only in the OR [t (78) = 2.637, p= 0.02] and SCC [t (78) = 2.519, p= 0.02].

The paired t-test results (FA, MD and RD) of the patients’ WM pathway changes as observed at 6 months (chronic phase) against the baseline (acute phase) values are presented in Table 4(a), 4(b) and 4(c), along with their effect sizes (Cohen’s d with <0.2 = small effect, >0.2 <0.8 = moderate effect and >0.8 = large effect). Various differences were seen in all DTI parameters, with greater alterations noted in the FA values across regions. Specifically, FA of the corona radiata (CR) [t (28) = 3.497, p=0.002, d=0.661], anterior limb of internal
capsule (ALIC) \(t(28) = 2.582, p=0.016, d=0.488\], CG \(t(28) = 2.973, p=0.006, d=0.562\], superior longitudinal fasciculus (SLF) \(t(28) = 2.404, p=0.024, d=0.454\], OR \(t(28) = 2.643, p=0.014, d=0.499\], and the genu of corpus callosum (GCC) \(t(28) = 2.732, p=0.011, d=0.516\] were significantly lower in the TBI group. Almost all of the MD values across the phases showed no significant changes except the CG \(t(28) = 3.189, p=0.004, d=0.603\] which was significantly higher in the TBI group. Although the changes in PLIC across the phases were found to be statistically non-significant \(t(28) = 1.494, p=0.15\], the effect size however suggests a moderate level of change \(d=0.282\). Finally, there were no significant differences in the RD values across the time points. However, Cohen’s \(d\) effect size calculation indicated moderate effect sizes for changes in the RD of CR \(t(28) = 1.582, p=0.126, d=0.299\], CG \(t(28) = 1.54, p=0.136, d=0.291\], OR \(t(28) = 1.87, p=0.073, d=0.353\] and GCC \(t(28) = 1.975, p=0.059, d=0.373\].

All the above stated significant differences were reflected in the TBSS images of the skeletonized tracts as well (see Figure 4).

**Neuropsychological Performance (NP)**

Table 5 presents the mean interpretive categories score comparison for the domain-specific NP among mTBI and healthy control groups. During the acute phase, patients with mTBI performed poorly across all domains in comparison to the healthy control group. The independent samples \(t\)-tests of both groups and their NPs indicated that the mTBI group was significantly poorer (all \(p\)-values \(< 0.001\)) on all but one of the neuropsychological domains (visuospatial functions: \(t(78) = 0.055, p=0.956\). Meanwhile during the chronic phase, patients with mTBI continued to perform poorly across most domains in exception of visuospatial functions where the patients outperformed the healthy controls \(t(48) = -2.373, p=0.021\).
Longitudinal analysis of WM tract changes against the NP at different intervals among a subset of the study patients (n=30) are presented in Table 6. Most of the observed FA associations with neurocognitive status (acute and chronic) represented negative associations. FA_a negatively correlated with the following acute NP_a: attention vs. CR (r = -.429, p<0.05), language vs. CR (r = -.375, p<0.05), language vs. SLF (r = -.557, p<0.01) and language vs. GCC (r = -.443, p<0.05). The acute FAs (FA_a) were also negatively correlated with the NP_c, as follows: language vs. MCP (r_s = -.440, p<0.05), attention vs. CR (r_s = -.441, p<0.05), language vs. CR (r_s = -.415, p<0.05), attention vs. SLF (r_s = -.417, p<0.05), language vs. SLF (r_s = -.409, p<0.05), spatial vs. CG (r_s = -.489, p<0.05). The two positive correlations seen in the chronic phase were the spatial vs. CG (r_s = .489, p<0.05) and spatial vs. SCC (r = .402, p<0.05). The significantly reduced FA_c had only two associations with the NP_c - language vs. CR (r = -.400, p<0.05) and spatial vs. SCC (r = .402, p<0.05).

MD_a showed limited associations with the NP_c. The following were the only MD_a values associated with the NP_c: attention vs. SLF (r_s = .404, p<0.05), spatial vs. CG (r_s = -.390, p<0.05) and spatial vs. GCC (r_s = -.404, p<0.05). No associations between the MD_c and NP_c were observed.

The RD of seven of nine tracts studied was significantly associated with attention, language, spatial and executive function in both phases of the study. Specifically, RD_a was associated with three NP_a scores: attention vs. CR (r = .485, p<0.05), attention vs. SLF (r = .487, p<0.05) and spatial vs. OR (r = -.378, p<0.05). Additional associations were later observed between RD_a and NP_c, which were as follows: language vs. MCP (r = .398, p<0.05), language vs. CR (r_s = .529, p<0.01), attention vs. SLF (r = .450, p<0.05), language vs. SLF (r = .491, p<0.05), language vs. CG (r = .423, p<0.05), spatial vs. CG (r = .626, p<0.01) and
attention vs. GCC ($r = -0.378$, $p<0.05$). Increased $RD_c$ values were also associated with some domains of the NP, including language vs. MCP ($r_s = 0.438$, $p<0.05$), executive function vs. CR ($r_s = 0.389$, $p<0.05$), executive function vs. CG ($r_s = -0.404$, $p<0.05$) and executive function vs. SCC ($r_s = -0.391$, $p<0.05$). No association was found between the domains of memory and any of the WM tracts investigated in this study. We also found no associations between the DTI parameters (FA, MD and RD) and any domains of the NP in the healthy control group.

**Neuropsychological Outcomes**

Table 7 presents the neuropsychological profiles of the subset of patients (n=30) who had completed neuropsychological evaluations as well as imaging both at admission and at the 6th month follow up. Within the domain of attention, 56.7% of the patients remained impaired at 6 months post trauma, 3.33% worsened, and the remaining improved or remained unaffected. Within the domain of language function, 63.3% of the patients remained impaired, 26.7% improved, 6.67% remained unaffected, and 3.33% worsened. Approximately 33.3% of the patients remained impaired within the domain of memory 6 months post-trauma with essentially equivocal changes (26.7% worsened and 26.7% remained unaffected, and 13.3% with improved memory status). The majority of the patients (53.3%) remained unaffected for spatial function although 23.3% showed signs of delayed impairments in the chronic phase. A total of 70.0% of the subset patient group remained impaired with respect to executive functioning, with only 20.0% of these patients showing signs of improvement or recovery after the 6-month period post trauma.

**DISCUSSION**

We examined the relationship between microstructural changes and neuropsychological functioning that takes place in the immediate aftermath of a mild head injury as well as six months post-TBI. Specifically, the initial neuropsychological assessment
was completed on an average of 4.35 hours following full GCS recovery, and the neuroimaging procedure was completed within an average of 10 hours post-trauma in order to identify the structural changes at a very early stage before they were subject to confounds such as changes specific to environmental and recovery parameters. Subsequently, both a repeat imaging and neuropsychological assessment were performed at an average of 6 months post trauma to characterize any pertinent changes over time.

Results showed associations between DTI and neuropsychological indices at both acute and chronic phases. Specifically, in the acute phase, the TBI group showed significantly poorer white matter integrity across several tracts, including the splenium (SCC), posterior limb of the internal capsule (PLIC), cingulum (CG), and optic radiation (OR). Several other white matter tracts beyond those initially affected also showed reduced FA at 6 months post-injury [i.e., corona radiata (CR), anterior limb of the internal capsule (ALIC), superior longitudinal fasciculus (SLF), and genu of the corpus callosum (GCC)]. Interestingly, moderate effect sizes for changes in RD were observed from baseline to follow-up in the following regions: CR, CG and OR. Finally, with respect to neuropsychological functioning, patients with mTBI performed more poorly across all domains in the acute stage when compared to healthy control participants. The majority of patients remained impaired with respect to executive functioning, and a sizable portion of the sample worsened over time on tasks of attention and language. Moreover, longitudinal analysis of WM tract changes as they relate to cognition showed several associations indicating that reduced white matter integrity was associated with specific neuropsychological decrements. Indeed, DTI indices in both the acute and chronic stages related to cognition at both time points, and RD values in seven of the nine tracts studied was significantly associated with attention, language, spatial and executive function in both phases of the study. Interestingly, no association was found between the domains of memory and any of the WM tracts investigated in this study.
Our findings of reduced FA, coupled with increased MD and RD in the acute phase, is considered indicative of vasogenic brain edema which refers to the release of intracellular proteins into the brain parenchyma, also known as extracellular edema. An increased FA, reduced MD, and RD in the acute phase, on the other hand, may be indicative of cytotoxic edema, also known as intracellular edema. This type of cerebral edema in the early course of the injury has been implicated in poor outcome, which could explain frequently observed neuropsychological impairment months or even years after the initial trauma event.

The increased FA, MD and rather an unchanged RD in the acute phase of this study is most likely indicative of reactive astrogliosis which is a process where fibrous astrocytes migrate to the site of injury, locally increasing the density of the cells and the diffusivity of the affected tissue.

Vasogenic edema occurs in the acute phase whereas demyelination occurs later. As such, even though the DTI markers for these pathogenic processes are the same, the timeframe of imaging changes rather indicate an ongoing vasogenic edema. These changes in DTI parameters were seen in the SCC, PLIC, CG and OR (with statistically significant changes in most parameters). Other tracts including the ALIC, SLF and GCC showed similar trends, although they did not reach statistical significance. Results showing minimally reduced FA in the context of significantly increased MD of the SLF and GCC in the patient group, and its negative association with language function, are similar to those reported by Ingelese et al and Arfanakis et al. Cognitive impairment observed acutely in this group of patients coupled with the changing DTI metrics are likely immediate signs reflecting an ongoing edematous process which is not observed via conventional CT or MRI.

In contrast, changes observed in CR and MCP were more suggestive of reactive astrogliosis and dovetails with findings recently reported by Croall and colleagues. Although some of these changes did not reach statistical significance, the effect size of 0.299
for the RD changes in CR, coupled with the effect size of 0.661 for the FA changes of CR, for instance, does provide some moderate to large evidence of significant change in this tract. Higher FA value of the CR also negatively correlated with attention and language functions acutely. Its corresponding positive association with RD at baseline best explains the influence of reactive astroglialosis on cognition in the acute stage as previously stated.\textsuperscript{40-42} The importance of the above finding cannot be understated as specific types cerebral edema and gliosis in the acute phase have been implicated to negatively influence long-term neuropsychological performance in patients with mTBI.\textsuperscript{43-46}

We also found that acute phase DTI parameters in selected WM tracts were significantly associated with chronic domain-specific cognitive deficits. This includes those of the MCP, CR, SLF, CG, GCC and SCC and their association with specific neurocognitive functions chronically, similar to findings of other studies.\textsuperscript{39-42, 47-48} Miles et al, for instance, noted that decreased FA values and increased MD values of the GCC, SCC and PLIC during the acute phase were significantly related to executive dysfunction at 6 months follow-up.\textsuperscript{2,47} Kumar et al and Matshushita et al reported similar findings involving the GCC, SCC and NP\textsubscript{c} in their studies of mild and moderate head injury.\textsuperscript{48-49} The mostly positive associations between the unchanged or raised RD\textsubscript{a} of the MCP, CR, SLF and CG with specific chronic deficits in attention, language and spatial function \textit{likely} reflect the long-term effects of acute vasogenic edema and reactive astroglialosis, a well-known immune response in the immediate aftermath of CNS injury.\textsuperscript{41} It is important to note that cerebral edema and cascading gliosis usually occur concurrently, and are not necessarily independent of each other.

Chronically, the continued alteration of DTI parameters especially the FA\textsubscript{c} and RD\textsubscript{c} implies significant changes in WM integrity, where the myelin sheath or the axonal membrane\textsubscript{c} or both\textsubscript{c} may have been permanently damaged.\textsuperscript{19-20, 36} This disruption \textit{may} be irreversible\textsubscript{c} especially when a reduced FA\textsubscript{c} and elevated MD\textsubscript{c} and RD\textsubscript{c} are noted.\textsuperscript{2,19}
Conversely, a reduced FA, unchanged MD, and subtly altered RD would mean that the microstructural changes to the WM are independent of gross tissue loss. The subtly altered RD in this context would imply minor fiber damage without gross tissue loss. Such changes have also been implicated as a part of the dual effects of gliosis.

FA measured at follow-up was significantly reduced in comparison to the acute phase with mostly unchanged MD and subtly increased RD across 6 tracts (i.e., CR, ALIC, CG, SLF, OR, GCC). While the changes seen in the ALIC and OR are indicative of possible minor fiber damage and thus vasogenic edema, the change pattern observed in CR suggests irreversible consequences of astroglialosis. The mixed association between certain neurocognitive performances in the chronic stage (e.g., executive, spatial and language function) and FA and RD of several tracts (i.e., MCP, CR, CG, and SCC), though paradoxical, is not completely unexpected. Similar observations were found in a recent study with attribution to the multifaceted chronic phase findings. This included neuronal network reorganisation, spurring of axons with smaller calibres, glial scarring, effects of accumulated phosphorylated neurofilaments and disruptive neurofibrillary tangles. Taken together, our findings further strengthen evidence for physiogenic influences on the prolonged functional and neuropsychological sequelae in patients with mTBI. Clearly, disruption of the connectomes (i.e., the connectivities between specific cortical and subcortical structures) by various neuropathological events post trauma and the recovery process over time requires further study.

Interestingly, a common finding in our study was that the more severely affected fibers were generally the long tract fibers (SLF, CR and CG) and commissural fibers (SCC, GCC). These fibers have an increased susceptibility to injury given their relatively long length and high membrane-to-cytoplasmic ratio. The corpus callosum, for example, as noted by Aoki and colleagues, is the major fiber bundle that enables communication between
the hemispheres and is topographically organized. The CC in general is divided into the genu (GCC), the body and the splenium (SCC). The CC has been long recognized as a frequently injured region due shear strain, topographical location and external accelerational forces can seriously injure the fiber. The posterior region of the CC, namely the SCC, often noted to be more vulnerable to injury than the anterior part (GCC), was replicated in this study as well and the SCC was the only tract that showed statistically significant changes in all three DTI parameters at the acute stage.

Our findings lend support to the theory that microstructural changes occur within hours after the initial insult and affect the integrity of the WM, therefore leading to the varied manifestation of neuropsychological impairments, in agreement with some of the earlier studies reported in the literature. Strengths of our study include examination within a short timeframe from time of injury to imaging and neuropsychological testing within the acute phase, and consistent test-retest interval at 6 month follow-up. Although FA and MD have been shown to be very sensitive in detecting subtle WM changes that correlate with neuropsychological findings, one other parameter that was included in this study, namely RD, provides further insight into the presumed nature of the microstructural changes, as it is sensitive to myelin integrity and also other CNS immune response such as gliosis. These parameters enabled us to detect the early deep white matter microstructural changes which might otherwise be confounded by ongoing secondary damage or recovery mechanisms. Additionally, the current study is unique in its longitudinal follow-up of DTI indices and cognitive functioning at 6 months post-trauma in a large cohort of well-characterized patients with relatively homogenous injuries. That said, findings may be limited due to the significant heterogeneity of the trauma observed in our sample as well as possible obscuring of certain differences due to the nature of brain morphing and averaging which is inherent in template-based analyses. Specifically, any deviation present in a small
number of voxels within a region may be hidden by the averaging and therefore lead to low sensitivity to detect FA and MD changes.\textsuperscript{81-82}

**Conclusion**

DTI is a useful technique used to assess the integrity of white matter tracts in patients who have sustained mTBI. This study found significant correlations between neuropsychological deficits and white matter tract integrity both within hours of neurotrauma and again 6 months later. These associations are likely attributable to alterations in the integrity of connectomes between specific cortical areas and subcortical structures, which is not evident in conventional MRI or CT procedures. Specific WM changes as observed through the DTI parameters especially in the acute period and the corresponding neuropsychological impairments among the patients in the chronic phase, highlights the need to introduce appropriate imaging techniques early in patient management protocols for early prognostication and rehabilitative intervention. Furthermore, our findings underscore the dynamic nature of brain injury and possible biologic basis of cognitive dysfunction in the context of the post concussive syndrome.

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References:


23. Sidaros, A., Engberg, A.W., Sidaros, K., Liptrot, M.G., Herning, M., Petersen, P.,
recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal

24. Arfanakis, K., Haughton, V.M., Carew, J.D., Rogers, B.P., Dempsey, R.J., and Meyerand,
Neuroradiol. 23: 794–802.

Based Diffusion Tensor Imaging Study of Uncomplicated Mild Traumatic Brain Injury in Adults. J
Neurotrauma 31: 466-475


imaging studies of mild traumatic brain injury: a meta-analysis. J Neurol Neurosurg
Psychiatry 83: 870–876.

28. Mukherjee, P., Miller, J.H., Shimony, J.S., Philip, J.V., Nehra, D., Snyder A.Z., Conturo,
matter development during normal human brain maturation. AJNR Am. J. Neuroradiol. 23:
1445–1456.


and reactive gliosis. Neuroscience letters, 565: 30-38.


