NEURONAL ACTIVATION IN PATIENTS WITH MILD AND MODERATE TRAUMATIC BRAIN INJURY DURING WORKING MEMORY TASKS

DR DIANA NOMA BINTI FITZROL

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LIST OF SYMBOLS, ACRONYMS AND ABBREVIATIONS

CTMT	Comprehensive Trail Making Test
DLPFC	Dorsolateral prefrontal cortex
EDH	Extradural haematoma
fMRI	Functional magnetic resonance imaging
HUSM	Hospital Universiti Sains Malaysia
MRI	Magnetic resonance imaging
mTBI	Mild traumatic brain injury
mTBI/ modTBI	Mild or moderate traumatic brain injury
modTBI	Moderate traumatic brain injury
PFC	Prefrontal cortex
RAVLT	Rey Auditory Verbal Learning Test
RCFT	Rey Complex Figure Test and Recognition Trial
SAH	Subarachnoid haemorrhage
SDH	Subdural haematoma
SMA	Supplementary motor area
TBI	Traumatic brain injury
USM	Universiti Sains Malaysia

VA/ DoD	United States Department of Veterans Affairs/ Department of Defense
VMPFC	Ventromedial prefrontal cortex
WASI	Wechsler Abbreviated Scale of Intelligence
WCST	Wisconsin Sorting Card Test
WM	Working memory

ABSTRAK

Tajuk

Pengaktifan neural pesakit-pesakit kecederaan otak traumatik tahap ringan dan sederhana semasa menjalankan activiti ingatan kerja.

Latar Belakang dan Objektif

Kemerosoton fungsi kognitif pesakit-pesakit selepas mengalami kecederaan otak traumatik adalah salah satu penyebab utama morbiditi di kalangan pesakit dan keluarga. Kerosakan memori merupakan salah satu ketidakupayaan yang sering dialami oleh pesakit dengan kecederaan otak traumatik. Modaliti pencitraan semasa membolehkan kajian teliti pengaktifan neuron bagi bahagian otak yang berkenaan dengan ingatan kerja. Kajian ini bertujuan untuk menyelidik bahagian otak yang berkaitan dengan fungsi ingatan kerja dalam pesakit dengan kecederaan otak traumatic menggunakan fMRI.

Kaedah

Kajian ini melibatkan pesakit dewasa, berumur 18 hingga 65 tahun, yang mengalami kecederaan otak traumatic yang ringan atau sederhana, dan dirawat di Hospital Universiti Sains Malaysia. Pesakit dan subjek kawalan akan menjalani ujian fMRI sambil melakukan aktiviti yang mempunyai beban atas ingatan pada tahap yan berlainan. Kedua-dua subjek juga akan menjalakan ujian neuropsikologi untuk menguji pemikiran kompleks dan daya

ingatan. Pemeriksaan fMRI dan neuropsikology dijalankan pada 6 hingga 10 minggu selepas trauma dan 6 bulan selepas trauma.

Keputusan

Kajian in melibatkan 7 pesakit dengan kecederaan traumatik otak and 9 subjek kawalan. Dari analisis, pada 6 hingga 10 minggu selepas kecederaan, pesakit dengan kecederaan otak traumatik didapati mempunyai aktivasi bahagian otak yang rendah berbanding dengan subjek kawalan. Bahagian otak yang telibat bertanggung jawab dalam fungsi ingatan kerja iaitu korteks prefrontal dorsolateral, korteks parietal yang rendah dan anting cingulate gyrus. Penemuan ini selari dengan keputusan dari analisis neuropsikologi. Pada 6 bulan selepas kecederaan otak traumatik, didapati bahagian kortex yang berlainan aktif di dalam otak pesakit-pesakit. Ini besar kemungkinan kerana proses neuroplasticity mengambil tempat semase tempoh pemulihan.

Kesimpulan

Penyelidikan ini mendapati bahawa pesakit dengan kecederaan otak traumatic mengalami kemerosotan ingatan kerja. Semasa peringkat awal fasa pemulihan, pesakit yang mengalami kecederaan otak traumatik tahap ringan atau sederhana mempamerkan penyusunan semula struktur dan fungsi kortikal yang mambantu dalam fungsi ingatan kerja. Peranan rehabilitasi semasa pemulihan amat penting.

ABSTRACT

Title

Neuronal activation in patients with mild or moderate traumatic brain injury during working memory tasks.

Background and Objective

Cognitive impairment in patients following TBI is a significant cause of morbidity both to patients and their families. Memory impairment is one of the most common complaint patients and their families have as it impairs patients' daily function. Current neuroimaging modalities allows us to study the neuronal activation relevant to working memory in patients.

Methods

Patients who sustained mTBI/ modTBI, between the age of 18 to 65 years old and managed in Hospital Universiti Sains Malaysia were recruited into the study. These patients and a control group underwent fMRI scanning where they concurrently performed *n*-back tasks of 4 different memory loads. These fMRI scans were performed at 6 to 10 weeks post-injury and at 6 months post-injury. These cohort of subjects also had neuropsychology assessments performed at the same two time period as the fMRI.

Results

At 6 to 10 weeks post-injury, patients with mTBI/modTBI exhibited much lower brain activation in the dorsolateral prefrontal cortex, inferior parietal gyrus and anterior cingulate gyrus compared to control which was consistent with the impairment of visuospatial WM. Neuropsychological assessments showed corresponding results for these patients. At 6 months after injury, other areas or the brain appeared to be active which may be due to underlying neuroplasticity.

Conclusion

Patients with mTBI/ modTBI are at risk of visuospatial WM dysfunction which may affect their quality of life. Hence the role of early and targeted rehabilitation for these patients during the recovery phase is crucial.

1.0. INTRODUCTION & LITERATURE REVIEW

Traumatic brain injury is a global health issue which causes long term disabilities to patients, burden to carers and increases the cost of the healthcare system (Rabinowitz and Levin, 2014). The incidence of TBI in 2016 in Malaysia was higher than the average in Southeast Asia and 2nd highest, behind Thailand (Collaborators, 2019). According to the 4th Report of the National Trauma Database published in 2009 by the Ministry of Health, males are 6.5 times more prone to trauma and younger age group of 15 to 34 years old have the highest risk (Jamaluddin *et al.*, 2009). Mild TBI (mTBI) is also more common than moderate (modTBI) or severe TBI (Jamaluddin *et al.*, 2009). These predispositions to TBI results in younger patients with mTBI/ modTBI who survive longer years with their disabilities compared to the elderly which further increases the demand on healthcare facilities (Collaborators, 2019).

One of the most common disabilities experienced by patients of all levels of TBI severity include neurocognitive deficits in particular impaired memory (Chen *et al.*, 2012). Patients with mTBI/ modTBI are prone to WM dysfunction which is fundamental to carry out daily activities and maintain a good quality of life (Smith *et al.*, 2015). WM is the ability to retain and manipulate certain amount of information when it is no longer present in the environment (Dobryakova, Boukrina and Wylie, 2015; Narayanan *et al.*, 2005). Current literature has identified a fronto-parietal network which is involved in WM specifically the dorsolateral (DLPFC) and ventromedial prefrontal cortex (VMPFC), the anterior cingulate gyrus and the posterior parietal cortex (Owen *et al.*, 2005; Wager and Smith, 2003).

The mechanism of TBI is associated with shearing injury to the axons which compromises the brain connectivity. The frontal and temporal lobes are most often affected

in TBI due to the close proximity to bony prominences and anterior location (Mckee and Daneshvar, 2015; Dunning, Westgate and Adlam, 2016). Hence patients with TBI including mTBI are prone to experiencing change in cognition including WM which can impact patients' and caregiver's lives significantly (McAllister, 2011; Narayana, 2017; Prince and Bruhns, 2017).

With current advances in neuroimaging it is possible to study the neuronal activation involved in WM of these patients. With better understanding we hope to be able to provide better targeted rehabilitation and management during the recovery process of patients with mTBI/ modTBI.

TITLE PAGE

Neuronal activation in patients with mild or moderate traumatic brain injury during working memory tasks.

MAIN AUTHOR : DIANA NOMA FITZROL[1]

AUTHORS : JAFRI MALIN ABDULLAH[1]

AINI ISMAFAIRUS ABD HAMID[1]

[1] DEPARTMENT OF NEUROSCIENCES, SCHOOL OF MEDICAL SCIENCES, UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN, KELANTAN.

Main Author Contact Details:

Department of Neurosciences,

School of Medical Sciences,

Universiti Sains Malaysia, Kelantan, Malaysia.

E-mail: diana_noma@hotmail.com

2.2. ABSTRACT

Background and Objective

Cognitive impairment in patients following TBI is a significant cause of morbidity both to patients and their families. Memory impairment is one of the most common complaint patients and their families have as it impairs patients' daily function. Current neuroimaging modalities allows us to study the neuronal activation relevant to working memory in patients.

Methods

Patients who sustained mTBI/ modTBI, between the age of 18 to 65 years old and managed in Hospital Universiti Sains Malaysia were recruited into the study. These patients and a control group underwent fMRI scanning where they concurrently performed *n*-back tasks of 4 different memory loads. These fMRI scans were performed at 6 to 10 weeks post-injury and at 6 months post-injury. These cohort of subjects also had neuropsychology assessments performed at the same two time period as the fMRI.

Results

At 6 to 10 weeks post-injury, patients with mTBI/modTBI exhibited much lower brain activation in the dorsolateral prefrontal cortex, inferior parietal gyrus and anterior cingulate gyrus compared to control which was consistent with the impairment of

visuospatial WM. Neuropsychological assessments showed corresponding results for these patients. At 6 months after injury, other areas or the brain appeared to be active which may be due to underlying neuroplasticity.

Conclusion

Patients with mTBI/ modTBI are at risk of visuospatial WM dysfunction which may affect their quality of life. Hence the role of early and targeted rehabilitation for these patients during the recovery phase is crucial.

Keywords: working memory, traumatic brain injury, mild TBI, moderate TBI, functional MRI

2.3. INTRODUCTION & LITERATURE REVIEW

Traumatic brain injury is a global health issue as not only does is cause long term disabilities to patients and burden to carers, it also stretches the healthcare resources and increases the expenditure due to the chronic requirements of patients (Rabinowitz and Levin, 2014). In addition, the loss of productivity and workforce also affects the economy negatively (Rabinowitz and Levin, 2014; Collaborators, 2019). The incidence of TBI in 2016 in Malaysia was reported to be 100, 399 which is roughly 5% of the incidence in Southeast Asia. However, age-standardized rates per 100,00 was 324 which was higher than the average in Southeast Asia and 2nd highest, behind Thailand (Collaborators, 2019). According to the 4th Report of the National Trauma Database published in 2009 by the Ministry of Health, males are 6.5 times more prone to trauma and younger age group of 15 to 34 years old have the highest risk (Jamaluddin *et al.*, 2009). Mild TBI (mTBI) is also more common than moderate (modTBI) or severe TBI (Jamaluddin *et al.*, 2009). These predispositions to TBI results in younger patients with mTBI/ modTBI who survive longer years with their disabilities compared to the elderly which further increases demand on healthcare facilities (Collaborators, 2019).

Cognitive impairments have been well-documented in literature to be one of the commonest morbidities affecting patients with mTBI/ modTBI in particular functions pertaining to attention and memory (Arciniegas, Held and Wagner, 2002; Chen *et al.*, 2012). With current advances in neuroimaging it is possible to study the neuronal activation involved in working memory of these patients. With better understanding we hope to be able to provide better targeted rehabilitation and management during the recovery process of patients with mTBI/modTBI.

2.3.1. Understanding the pathophysiology of TBI

It is essential to understand the nature and basic pathophysiology of TBI to be able to put into context the changes following such an event (Medaglia, 2017). Menon et al, on behalf of The Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury and Psychological Health, have defined TBI as an alteration in brain function or other evidence of brain pathology caused by an external force (Menon *et al.*, 2010). This refers to external mechanical forces to the brain that leads to either permanent or temporary alterations in cognitive, physical and psychosocial functions.

Mechanism of injury to the brain can be divided into primary and secondary brain injury (Prins *et al.*, 2013). Primary brain injury refers to the immediate injuries due to direct trauma to the head which includes structural changes such as tissue distortion, axonal shearing and vascular injury. Primary injury can be categorized into closed or blunt injury, penetrating injury and blast injury (Zollman, 2016). Mechanisms of closed head injuries include direct force to the head, acceleration-deceleration injury and rotational forces. Blast injuries are due to pressure waves generated from explosives which emit thermal, mechanical and electromagnetics energy (Zollman, 2016). Penetrating injuries are injuries where the dura is breached and this can be further subdivided into low-velocity and high velocity injuries (Alao and Waseem, 2019). Penetrating head injuries include gunshot wounds, shrapnel and knife wounds (Zollman, 2016).

In actual real time, brain injuries are usually a result of a combination of mechanisms hence are usually different in each case depending on the locations involved, types and extent of injuries sustained. However, despite these difference, the basic molecular changes within

the brain that ensue the initial trauma are similar and cause further neurovascular damage which underlie the second phase of TBI (Medaglia, 2017). Secondary brain injury is a constellation of dynamic processes which include ischaemia, inflammation and cytotoxic changes (Veenith *et al.*, 2016). Tissue damage from these occur due to generation of free radicals, disruption of ionic homeostasis and blood-brain barrier, mitochondrial dysfunction, axonal disruption, apoptosis and lipid peroxidation (Prins *et al.*, 2013; Zollman, 2016).

In contrast to primary brain injury which usually occurs in a matters of milliseconds, secondary brain injury usually worsens over 24 to 48 hours and this is the time period where medical and surgical management plays a pivotal role in patient care (Kaur and Sharma, 2018). There is no fixed time as to the recovery process for patients following TBI, however the fastest recovery to occur is within the first 6 months and may continue even years after injury (Bernier and Hillary, 2019). During this recovery phase, the process of neuronal plasticity where neuronal re-organization and recruitment occurs (Bernier and Hillary, 2019).

2.3.2. Classification of TBI

TBI can be classified based on clinical and radiological features. The most common classification used in clinical setting is based on the Glasgow Coma Scale (GCS) which has a score of 3 to 15 and differentiates the severity of TBI into mild (GCS score 13 to 15), moderate (GCS score of 9 to 12) and severe (GCS score of 3 to 8). These scores are based on patients' eye, verbal and motor responses (**Table 1**). Patients with TBI are assessed on presentation following the traumatic event.

The United States Department of Veterans Affairs in collaboration with the Department of Defense (Va/DoD) has developed a clinical practice guideline for TBI utilizing other clinical parameters such as loss of consciousness (LOC), alterations in consciousness or mental state (AOC) and post-traumatic amnesia (PTA) and radiological features when classifying severity of TBI (**Table 2**)(O'Neil *et al.*, 2013). Based on this classification, if a patient fits more than one of the criteria, the higher severity of TBI is assigned.

2.3.3. Working memory in patients with TBI

Patients with mTBI/ modTBI are prone to long term cognitive impairment in particular WM which is fundamental to carry out daily activities and maintain a good quality of life (Smith *et al.*, 2015). WM is defined as the process of retaining and manipulating certain amount of information when it is no longer present in the environment over a short period of time (Dobryakova, Boukrina and Wylie, 2015; Narayanan *et al.*, 2005). This ability to hold information in memory for a brief period is pivotal in cognitive functions such as language, planning, decision making and abstract thoughts (Constantinidis and Wang, 2004; Murray, Jaramillo and Wang, 2017). Current literature has identified a fronto-parietal (Jensen and Tesche, 2002) network which is involved in visuospatial WM specifically the dorsolateral prefrontal cortex (DLPFC), ventromedial prefrontal cortex (VMPFC), the anterior cingulate gyrus and the posterior parietal cortex (**Figure 1**) (Owen *et al.*, 2005; Wager and Smith, 2003; Huang *et al.*, 2019). During visuospatial WM, the right DLPFC receives input from the right posterior parietal cortex and dorsal visual stream which is involved in processing of visuospatial information (Constantinidis and Wang, 2004; van

Asselen *et al.*, 2006). The right posterior parietal cortex appears to play a role in remembering the location of the visual stimuli (Constantinidis and Steinmetz, 2005). Studies have demonstrated that the anterior cingulate gyrus has higher activation during high memory load tasks and shown to help maintain attention and retain memory (Duma *et al.*, 2019). It has also been demonstrated that during visuospatial WM tasks, other areas of the brain such as the premotor cortex, SMA, superior colliculi, basal ganglia and cerebellum are also active (Zanto and Gazzaley, 2019; Barbas *et al.*, 2018; Luu *et al.*, 2014).

The frontal and temporal lobes are particularly prone to injury in a traumatic event due to its large volume which makes it susceptible to rotational shearing and close proximity to bone prominences (Clark, Boutros and Mendez, 2018). Shearing injury to the axons subsequently compromises brain connectivity within the frontotemporal region. (Mckee and Daneshvar, 2015; Dunning, Westgate and Adlam, 2016). Hence patients with TBI including mTBI are prone to experiencing change in cognition including WM which can impact patients' and caregiver's lives significantly (McAllister, 2011; Narayana, 2017; Prince and Bruhns, 2017). The anterior cingulate gyrus, although medial is also prone to injury during trauma due to its proximity to the bone, superoanterioly, and falx, medially, which tend to be more rigid in its anterior aspect (Merkley *et al.*, 2013). mTBI was traditionally assumed to be a transient disruption however has been shown to cause long term structural change to the brain parenchyma (Povlishock, 1993).

2.3.4. Recovery phase following TBI

Long term structural changes following TBI include axonal and myelinic changes. These demyelination process and loss of axons have been suggested to be responsible for the irreversible damage in TBI which contributes to cognitive deficits (Kraus *et al.*, 2007). It is thought that during the recovery phase, patients tend to experience some degree of improvement which thereafter plateaus (Povlishock and Katz, 2005; Christensen *et al.*, 2008). However, there are contradicting evidence from studies which reported cognitive decline in patients with TBI in their chronic stages of recovery which were 1 to 2 years after their traumatic events (Masel and DeWitt, 2010; Himanen *et al.*, 2006). These were attributed to chronic structural changes which were brain atrophy, loss of white matter integrity and gliosis that can occur up to 2.5 years post-injury (Adnan *et al.*, 2013; Johnson *et al.*, 2013; Farbota *et al.*, 2012). Farbota et al. demonstrated in their study, where they followed up with their cohort of patients for 4 years, that there were changes in the corpus callosum consistent with reduced connectivity which were accompanied by compensatory changes in the white matter tracts through the superior longitudinal fissures. However, these changes were not significant enough to manifest in the neuropsychology assessments (Farbota *et al.*, 2012). Rehabilitation programmes during the recovery phase of chronic TBI patients have been reported to be of benefit to reduce the cognitive and behavioural deficits (Tomaszczyk *et al.*, 2014; Chiaravalloti *et al.*, 2015). The underlying principle of neurorehabilitation appears to be neural plasticity where brain connectivity is able to re-organize and allow re-education of certain skills by the patients (Lemmens *et al.*, 2013; Faralli *et al.*, 2013; Benowitz and Carmichael, 2010).

Training of WM following TBI has recently been studied however evidence on benefit and outcome of long-term post-traumatic interventions is still lacking (Nygren-de Boussard *et al.*, 2014). Recent studies and ongoing trials are yet to be conclusive (Vallat-Azouvi and Azouvi, 2019; Barman, Chatterjee and Bhide, 2016; Krawczyk *et al.*, 2019). It is hoped that with better understanding of the natural history of cognitive function in patients with TBI, a more targeted rehabilitation therapy focusing on different aspects of neurocognition and time period during the recovery phase can be established (Tomaszczyk *et al.*, 2014).

Current literature on cognitive dysfunctions following TBI are either more focused on moderate and severe TBI, understandably due to gross cognitive dysfunction, or study patients with TBI as one general group (Oberholzer and Müri, 2019; Königs *et al.*, 2018). Hence, in this study, we focused on mild and moderate TBI as these cohort of patients are the majority of TBI patients and are more likely to go undiagnosed despite being affected by their condition (Kalechstein, Newton and van Gorp, 2003; Prince and Bruhns, 2017).

2.4. OBJECTIVES

2.4.1. To identify regions of brain activation during WM tasks using fMRI in patients with mTBI/ modTBI as compared to controls during the recovery phase at 6 to 10 weeks and at 6 months after traumatic event

2.4.2. To correlate the fMRI findings of neuronal activation involved in WM of patients with mTBI/ mod TBI with neuropsychological assessments at the corresponding time periods.

2.5. MATERIALS AND METHODS

2.5.1. Patients with mTBI/ modTBI and controls

This study was carried out in Hospital Universiti Sains Malaysia (HUSM) which is a tertiary hospital in the east coast of Malaysia. This was a prospective study and received ethical approval by Human Research Ethics Committee USM (**Appendix A**). Patients with mTBI/ modTBI were identified via the local referral system. Controls were recruited through volunteers. Recruitment into the study was discussed with the patients who were eligible and written consent was obtained from either the patients or their main carers. Subjects who did not meet the inclusion criteria or had any of the exclusion criteria were not included in the study (**Table 3** and **Table 4** respectively).

The requirement to have 9 years of education is based on the Malaysian education system whereby subjects and controls have completed primary school and at least half of

their secondary school education which is up to 15 years old. This is to standardized the level of education among the subjects as to avoid confounding bias (Pliatsikas *et al.*, 2019). Those recruited were also required to be able to understand and abide the instructions given during the fMRI procedures without any underlying conditions or external influences which may affect their working memory performance and accuracy of the results.

Patients with mTBI/ modTBI recruited underwent fMRI scanning with assigned tasks and neuropsychology assessments at 6 to 10 weeks after trauma (acute phase of recovery) and at 6 months after trauma (subacute phase of recovery)(Institute of Medicine . Committee on Cognitive Rehabilitation Therapy for Traumatic Brain *et al.*, 2011). Controls underwent fMRI scanning with the same tasks and neuropsychology assessments as performed on the subjects with mild or moderate TBI.

2.5.2. fMRI data acquisition

Recruited patients underwent fMRI scanning in the Radiology department in HUSM. The MRI machine in HUSM was a Philips Achieva 3.0T scanner. T1-weighted structural images were acquired using magnetization prepare gradient echo (MPRAGE) sequence: 256 x 256 matrix, 160 sagittal slices, repetition time/ echo time/ flip angle (TR/ TE/ FA) = 2600 ms/ 3.05 ms/ 8◦, final resolution= 1 x 1 x 1 mm3. Functional T2-weighted images were acquired using a 32-channel head coil with a gradient echo single-shot echoplanar sequence: TR/ TE/FA= 2000 ms/30 ms/90°, field of view = 240 × 240 mm, matrix= 80 × 80, 37 oblique slices (parallel to orbitofrontal cortex to reduce sinus artifact), slice thickness= 2.5 mm with 0.5 mm gap, interleaved slice acquisition.

Subjects performed the working memory tasks while lying supine in the scanner. Foam cushioning and tape were used to immobilize the head within the coil to minimize motion degradation. Visual stimuli were presented to subjects through MRI compatible screens.

2.5.3. fMRI and *n*-back tasks

To assess the WM, we utilized the *n*-back tasks where participants were presented with a series of alphabets stimuli (Gevins and Cutillo, 1993; Uddin *et al.*, 2017; Jacola *et al.*, 2014). Participants received visual and verbal instructions on how to perform these tasks. They were informed to respond to whenever an alphabet stimulus presented was the same as that presented *n* back previously. White alphabets were displayed on a black background as stimuli.

There were 4 conditions that were included in the *n*-back tasks (0-back, 1-back, 2-back, 3-back). The 0-back condition required minimal working memory load with increasing difficulty as participants progressed to 1-back, 2-back and 3-back respectively. In 0-back task, participants were asked to respond by pressing a button provided if the alphabet displayed matched the previous alphabet they had just seen. In 1-back task, participants were to respond if the alphabet they saw matched the alphabet they had seen prior to 1 previous alphabet. In 2-back and 3-back tasks, similarly, participants had to respond if the alphabet they saw matched prior to 2 and 3 previous alphabets respectively. In each condition there were 6 blocks and 10 trials were run for each block. Participants viewed a continuous string of 10 images with 1 target stimuli at a time on a computer screen for 500 ms with 1500 ms

inter trial interleave (2 seconds each picture), onset to onset. There were two targets in each block. The total duration of the stimulus was 16 minutes.

2.5.4. fMRI data analysis and processing

Data was analyzed for neural activation using MATLAB R2019a (MathWorks Inc., Natick, MA, USA) and Statistical Parametric Mapping (SPM9)(Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College of London, UK; http://www.fil.ion.ucl.ac.uk/spm). The first two volumes of functional scans were discarded.

Pre-processing of data was performed with SPM. Slice timing (1:6:35 2:6:35 3:6:35 4:6:35 5:6:35 6:6:35, slice reference 33)(Sladky *et al.*, 2011) and realignment to reduce head motion correction, co-registration of the functional with anatomical images, segmentation, normalization to a template at the Montreal Neurological Institute (MNI) space and smoothing with an 8mm Gaussian kernel to reduce the signal-to-noise ratio was performed (voxel size 1.7 x 1.7 x 3)(Friston *et al.*, 2000).

fMRI analysis included statistical parametric mapping using a general linear model as implemented in SPM9. Smoothed normalized data scans for participants were entered into the model, and contrasted images comparing the block designs and subjects against controls were created.

Analysis of region activation were focused on the frontal, temporal and parietal regions as these are the regions of the brain prone to injury and involved in WM.

2.5.5. Neuropsychology assessments

The neuropsychology assessments were performed by a neuropsychologist in HUSM at the same time period that each participant underwent the fMRI scan.

The neuropsychology assessments were performed to test higher cognitive functions relevant to working memory performance and these include Wechsler Abbreviated Scale of Intelligence (WASI), T-score block design and T-score matrix reasoning (subsets of WASI), Rey Auditory Verbal Learning Test (RAVLT) immediate and delayed memory, Wisconsin Sorting Card Test (WCST), Rey Complex Figure Test and Recognition Trial (RCFT) immediate and delayed recall and Comprehensive Trail Making Test (CTMT).

WASI tests the overall and specific cognitive abilities, suitable for ages 6 to 89 years old. It consists of 4 subtests which are Vocabulary, Block Design, Similarities and Matrix Reasoning (McCrimmon and Smith, 2013). RAVLT immediate and delayed memory assesses a subject's verbal memory. It is designed as a list-learning paradigm test and subject listens to a list of 15 nouns (List A) and is asked to repeat as many as he or she could up to 5 times with 5 repetition of free-recalls of the List A. The subject then hears another list of nouns (List B) and asked to repeat the task. Following that, the subject is then again requested to recall nouns from List A and a third time 20 minutes later (Bean, 2011). WCST assesses one's ability for abstract thinking and planning in a dynamic environment. Subject is given a set of cards with instructions on how to match these cards (Kohli and Kaur, 2006). RCFT immediate and delayed recall tests for the subject's visuospatial ability and visuospatial memory. The subject is asked to view and draw free-hand a complex figure

whilst viewing, at 3 minutes (immediate memory) and at 30 minutes (delayed memory) (Gallagher and Burke, 2007). CTMT comprises of 5 visual search and sequencing task sets. This tests assess the subject's attention, psychomotor speed, concentration, resistance to distraction and cognitive flexibility (Moses, 2004).

The International Business Machine Corporation (IBM) Statistical Package for the Social Sciences (SPSS) Statistics version 23 software was used to analyse the results from the neuropsychology assessments. Independent T-test was performed to find statistically significant difference between the neuropsychology assessments of patients with mTBI or modTBI and controls. Paired T-test was used to compare the results of patients with mTBI or modTBI at baseline (6 to 10 weeks after trauma) and 6 months after trauma.

2.6. RESULTS

There were 47 patients with mTBI/ modTBI who were eligible to participate in the study. 37 agreed and consented however 4 further decided to drop out from the study prior to first fMRI scan and further 2 dropped out after first fMRI scan. Due to technical difficulties with the fMRI machine, 7 patients underwent fMRI at 6 to 10 weeks after trauma and 3 underwent fMRI at 6 months after trauma. These patients also had neuropsychology assessments performed at the same period they underwent fMRI scanning. All patients were Malay male adults (Table 5). There were 9 Malay male adults in the control group. All participants in the study were right-handed. The demographic backgrounds of controls were matched with patients with mTBI/ modTBI (**Appendix B**).

2.6.1. fMRI and *n*-back tasks performances

Group analysis performed with SPM9. Statistical parametric map calculation was based on the voxel-by-voxel method, using a general linear model. Contrast images for differences in activation between the healthy controls and TBI patients were created. The probability threshold was set at $p<0.001$ uncorrected, $k>10$. The MNI coordinates were then used in conjunction with WFU_PickAtlas 3.0.5 (Wake Forest Pickatlas) and aal (Automated Anatomical Labelling) to identify the anatomical landmarks (Maldjian *et al.*, 2003; Maldjian, Laurienti and Burdette, 2004; Tzourio-Mazoyer *et al.*, 2002).

Contrast images for patients and controls undergoing all the 4 levels of memory loads were generated and regions activated that were involved in working memory were compared.

The contrasted images (**Figure 3, Figure 4**) demonstrated that there were more regions activated in controls with higher voxel peaks during all the 4 levels of memory loads as compared to patients with mTBI/ modTBI (**Table 6**).

From **Table 6**, we can identify the consistent regions of the brain that were significantly activated during most of the working memory tasks for the patient and control groups. These include the left supplementary motor area (SMA), left superior or middle frontal gyrus, right middle frontal gyrus, left inferior parietal gyrus and right inferior parietal gyrus. In these regions, the patterns generally showed higher voxel activations in control groups as compared to patients with mTBI/modTBI. However, except for the left inferior parietal gyrus, the general pattern in the control group showed higher voxel activation with increasing memory loads. The reverse is seen in patients with mTBI/ modTBI where there was lower voxel activation with higher memory loads in the regions activated.

The right pars opercularis, right pars triangularis and right pars orbitalis which are part of the right inferior frontal gyrus appear to be activated in the control group during 3-back task as compared to the patients with mTBI/ modTBI where these regions were activated during 0-back task. The pattern of activation of these 3 regions in controls during 0-back to 2-back tasks are seen similarly in patients with mTBI/ modTBI but during 1-back to 3-back tasks.

The right angular gyrus was activated in patients with mTBI/ modTBI whilst performing all 0-back to 3-back tasks, but only in controls when performed low memory load task, 0-back. The right middle cingulate gyrus, left insula and right insula was activated only in higher memory load tasks in controls but not patients with mTBI or modTBI.

The peak voxels in controls appear to be higher with high memory load tasks however the opposite seems to be applicable in patients with mTBI/ modTBI.

At 6 months post-trauma, the 3 patients who underwent fMRI showed more regions of activation as compared to baseline (**Figure 5**).

Regions involved in working memory which was significantly activated in patients at 6 months post trauma as compared to regions at 6 to 10 weeks after trauma are listed as in **Table 7**. We can see that there are more regions activated after 6 months of trauma than in the period right after trauma.

At 6 months post-trauma, patients with mTBI/modTBI showed peak activations in some regions that were not significantly activated in controls and in patients with mTBI/modTBI at 6 weeks post-trauma. These regions include bilateral thalamus, the left

pars orbitalis, left pars triangularis, left pars opercularis, left superior temporal gyrus and right amygdala.

The left middle frontal gyrus, right middle cingulate gyrus, right middle temporal gyrus and bilateral putamen were regions significantly active in controls but not in patients with mTBI/modTBI at 6 weeks post-trauma.

At 6 months after injury, the left sup frontal gyrus, right putamen and right middle temporal gyrus appear to have higher voxel activation as compared to controls.

When neuronal activation in patients at 6 to 10 weeks after injury were compared to 6 months after injury to assess significant areas activated at baseline, there were no significant areas found (**Appendix C**).

2.6.2. Neuropsychology assessments

The neuropsychology assessments in patients with mTBI/ modTBI (**Table 8**) showed generally lower scores than controls (**Table 9**) except for Patient 2 who have scored either average or above average at each assessment. Unfortunately, this patient did not have a further assessment at 6 months as he opted to drop out from this study.

Table 8 shows that not one assessment was specifically affected however the WCST test, RCFT immediate and delayed recall and CTMT test appeared to be more poorly scored by all the patients with mTBI/modTBI except for patient 2.

From **Figure 6**, we can see that taking p-value < 0.05, there were a significant differences in the performances of patients with mTBI/modTBI as compared to controls in

WASI, T-score Matrix reasoning, RAVLT immediate and delayed memory and RCFT immediate recall assessments. RCFT delayed recall had a p-value of 0.55 which almost reached statistical significance.

Table 10 showed improvement in results of the 3 patients with mTBI/modTBI although not in any particular assessment. Patient 1 showed improvement in WASI, T-score matrix reasoning and RCFT immediate and delayed recall tests. Patient 3 had improvement in RAVLT immediate and delayed memory, WCST and RCFT immediate and delayed recall. Patient 5 had improvement in T-score Matrix Reasoning, WCST, and RCFT immediate and delayed recall assessments. **Figure 7** shows that there was significant improvement in T-score matric reasoning and RCFT delayed memory test.

2.7. DISCUSSION

WM helps us to retain certain information for a limited period of time, usually seconds to minutes for goal-orientated behaviour (Eriksson *et al.*, 2015). Brain regions involved in WM differ according to the type of information received. Studies have shown that lesions in temporal region tend to affect visual WM and lesions in the parietal region affect the spatial WM. The lateral prefrontal cortex (LPFC) also have been shown in anatomical studies to have connections with the parietal cortex and temporal cortex, specifically from the dorsal LPFC and the ventral LPFC respectively (D'Esposito *et al.*, 1998).

6 out of 7 of the patients in this study had clinical and radiological evidence of injury to either the temporal or frontal or both regions. The DLPFC is a functional anatomical site

instead of a structural site. The DLPFC is consistent with Brodmann area (BA) 8, 9 and 46 which comprises the middle frontal gyrus and part of the superior frontal gyrus. The DLPFC is bordered by the inferior frontal sulcus (inferolateral), paracingulate gyrus (medial), precentral sulcus (posterior) and frontal pole (anterior) (Sanches *et al.*, 2009). The posterior parietal cortex responsible for attention in visuospatial WM consists of the intraparietal sulcus and inferior parietal gyrus (Malhotra, Coulthard and Husain, 2009). The superior parietal gyrus is involved in the dorsal visual pathway. In our cohort of subjects, both the controls and patients with mTBI/ modTBI had peak activation of the right middle frontal gyrus which corresponds to the DLPFC and right inferior parietal gyrus corresponding to the posterior parietal cortex. The DLPFC in controls were activated at higher voxels with increasing memory load and the opposite trend was observed in patients with mTBI/ mod TBI. The right posterior parietal cortex was also consistently activated in both groups however much higher in the control groups. Chen *et al* who studied neuronal activation in patients with mTBI demonstrated in his study where healthy controls had increased neuronal activation with increasing memory loads but there was lack of increased neuronal activation with increasing memory load in patients with mTBI which was similar to our findings (Chen *et al.*, 2012). Studies previously have had inconsistent fMRI findings where by some have demonstrated increased activation in patients with TBI and some have demonstrated decreased activation in patients with TBI (McAllister *et al.*, 2001; Stubbs *et al.*, 2019; Chen *et al.*, 2012; Chen *et al.*, 2004; Sanchez-Carrion *et al.*, 2008). These discrepancies may be due to differences in patient demographics, varied trauma mechanisms and different time periods when the studies were carried out. The initial extent and severity of injury in focal

and diffuse TBI which varies may have also contributed to degree of neuronal activation in patients (Sanchez-Carrion *et al.*, 2008).

The right middle cingulate gyrus, which also known as part of the dorsal anterior cingulate gyrus, was demonstrated in our cohort to be significantly active only in the control groups throughout all the 4 *n*-back tasks however no neuronal activation was detected in patients with mTBI/ modTBI (Jumah and Dossani, 2019). This region appears to involved in memory and attention-based tasks (Lenartowicz and McIntosh, 2005). In a study by Merkley et al., they demonstrated specific regional atrophy of the right dorsal anterior cingulate gyrus in patients with severe TBI at 2 to 18 months post-injury which was accompanied with worse neurocognitive assessments (Merkley *et al.*, 2013). Another study reported atrophy of bilateral anterior cingulate gyrus after 1 year post-injury in patients with mTBI (Zhou *et al.*, 2013). In this study by Zhou et al, the patients did demonstrate worse neurocognitive performance compared to controls, but they did score significantly higher levels on scales of depression.

Although there is lower activation of the right posterior parietal cortex in patients with mTBI/ modTBI, there is activation of the right angular gyrus in these patients which was not present in the control groups. It is possible that this may represent map expansion of the functional area for posterior parietal cortex, one of the mechanisms underlying neuroplasticity which involves recruitment of the adjacent normal functional regions to function during the intended tasks (**Figure 8B**)(Sanchez-Carrion *et al.*, 2008).

In fact, regions of the brain activated in patients with mTBI/ modTBI at 6 months post-injury (**Table 7**) that were absent at 6-weeks post-injury likely represents the process of neuroplasticity which allowed for compensatory mechanisms for patients to perform