Magnetic Resonance Spectroscopy Imaging of the Effects of treatment of TBI Depression: Lessons Learned

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Abstract

Background: Major depression is a common complication following traumatic brain injury. It is associated with poor global clinical and psychosocial outcomes. Even though there are several studies on risk factors for major depression after TBI (henceforth will be referred to as TBI depression) there is minimal literature on the treatment of TBI depression. Further, the neurobiological changes associated with treatment have not been a focus of study.

Aims: (1) To determine the effectiveness of escitalopram for the treatment of TBI depression as assessed by the Montgomery Depression H1 or proton before rating Scale (MADRS) and Clinical Global Impression-Improvement (CGI-I) scale. (2) To determine differences in brain metabolite ratios as measured by single voxel magnetic resonance spectroscopy (MRS) and multi slice magnetic resonance spectroscopy imaging (MRSI) before and after treatment with escitalopram.

Methods: Double-blind, randomized controlled trial. Subjects were randomized to either the escitalopram or the placebo group. Escitalopram was started at 10mg per day and increased to 20 mg if deemed clinically necessary, at week 4. No medication changes were made after week 8. The placebo group received a pill which appeared similar to the 10 and 20 mg of escitalopram. All subjects had a total of 4 visits: baseline evaluation and follow-up visits at weeks 4, 8 and 12. All subjects underwent brain MRS/MRSI scans before (Week 1) and after treatment (week 12).

Results: At the end of 12 weeks of intervention, no statistically significant differences were found between the drug and placebo groups on the MADRS or the CGI-I scale scores. There were also no statistically significant differences between the two groups on the brain metabolite ratios. However, there was a trend towards statistical significance (p value =<0.1) in escitalopram compared to placebo group after treatment. In the escitalopram compared to placebo groups, changes before to after treatment were observed. Higher Cho/Cr ratios in the right frontal cortex (median values: -0.08 versus 0.13, p value =0.08), higher NAA/Cr ratios in the right thalamus (median values: -0.12 versus 0.19 p-value=0.09), higher NAA/Cho ratios (median values: -0.40 versus -0.02, p-value = 0.06), lower NAA/Cr ratio (median values: 0.28 versus -0.38; p-value = 0.06) and lower Cho/Cr ratio (median values: 0.23 versus -0.09; p-value = 0.06) in the left frontal cortex.

Conclusion: These preliminary findings support the further investigation of changes in brain metabolites before and after treatment with antidepressants using larger sample sizes and strategies to reduce or eliminate artifacts in magnetic resonance imaging. Such neuro imaging studies have the potential to provide an understanding of biological mechanisms of TBI depression.

INTRODUCTION

Depression is a frequent and debilitating consequence of TBI [1]. There is no FDA approved medication for the treatment of TBI depression [2]. Selective serotonin re-uptake inhibitors (SSRIs) have the best preliminary evidence [3,4] even though some have failed to show a positive response [5].

Magnetic resonance spectroscopy imaging (MRSI) is a non-invasive technique to record human and animal biochemistry in vivo [6]. Single voxel proton magnetic resonance spectroscopy (MRS) and multi slice magnetic resonance spectroscopy imaging (MRSI) clinical uses range from disorders in newborns to dementia in elderly, for diagnostic and therapeutic monitoring [6,7]. Some of the uses of MRSI includes differentiating low grade gliomas from infarction in young stroke patient, improving diagnostic sensitivity in identifying hippocampal sclerosis in patients with temporal lobe epilepsy with absent or equivocal magnetic resonance imaging (MRI) findings, predicting who will develop Alzheimer’s dementia in patients with mild cognitive impairment [8] and even determining the effects of neuro degeneration in patients with primary open glaucoma [9].

Brain metabolites that can be identified with MRS are N-acetylaspartate (NAA), choline (cho), creatinine (cr), possibly...
alanine and lactate at intermediate to long echo time and myo-inositol, glutamine and glutamate, glucose at short echo times [8]. N-acetylaspartate (NAA) is a marker of neuronal density and viability [8] and may be an indicator of neuronal and axonal integrity [10]. N-acetylaspartylglutamate (NAAG) appears to be involved in excitatory neurotransmission and in glutamate synthesis [11] NAA and NAAG are commonly expressed as a combined measure because of their overlapping peaks and are considered to be markers of healthy and viable neurons [12]. Choline (Cho) is a metabolic marker of membrane density and integrity [8]. It is a product of cell membrane metabolism. Increased Cho levels suggest cell membrane breakdown or cell proliferation [10]. Creatine (Cr) and phosphocreatine are involved in energy metabolism and their levels are decreased in conditions associated with cell death [10]. Amino acids glutamates (Glu), glutamine (Gln) are regulators of neuronal excitation and inhibition and have been implicated in the pathogenesis of mood disorders [13].

Neuroimaging studies in the evaluation of the neurobiological effects of pharmacological treatments are important as they could provide objective markers of the impact of such treatment. Studies using MRSI and Proton Emission Tomography (PET) methods in idiopathic major depressive disorder have shown that effective treatment with SSRIs is associated with increased glucose metabolism and neurochemical changes such as increased NAA/Cr values in the anterior cingulate cortex and medial prefrontal cortex [14-16]. Proton magnetic resonance spectroscopy (MRS) studies have also shown reduced glutamine levels after treatment with lamotrigine in patients with non-melanocholic bipolar depression [17]. These studies suggest that treatment with anti depressants or mood stabilizers might play a positive role in restoring neuronal function.

In two earlier studies using MRSI, we noted that patients with TBI depression in comparison to normal controls had significantly reduced N-acetylaspartate/choline (NAA/Cho) and NAA/creatine (Cr) ratios in frontal cortex, basal ganglia and thalamus [18]. Subjects with TBI depression compared to TBI non-depression had lower Cho/Cr and NAA/Cr ratios in the right basal ganglia [19].

To date, no studies have assessed whether brain metabolic changes may be predictive or correlated with response to antidepressant treatment for TBI depression. Given the well-described therapeutic effects of SSRIs in idiopathic depression and the role of the serotonin system in TBI [3,4,20], We conducted a double-blind placebo controlled pilot study to determine the brain metabolic effect of escitalopram in TBI-related depression. We have used the term 'TBI depression' broadly, to include development of major depression any time after traumatic brain injury.

**HYPOTHESES**

**Primary**

We hypothesized that following 12 weeks of treatment with escitalopram, participants in the escitalopram group, when compared to those in the placebo group would demonstrate:

a) Statistically significant lower scores on the Montgomery Depression Rating Scale (MADRS)
b) Statistically significantly higher rates of improvement on the Clinical Global Improvement (CGI-I) scale and
c) Statistically significantly higher N-acetylaspartate / Choline (NAA/Cho) and higher NAA/creatine (NAA/Cr) ratios in the frontal cortex, hippocampus, thalamus, and basal ganglia and statistically significantly higher NAA+NAAG/Cr and lower Glu+Gln/Cr in the hippocampus.

**Secondary**

We hypothesized that following 12 weeks of treatment with escitalopram, participants in the escitalopram group, when compared to those in the placebo group would demonstrate statistically significant lower scores on the Clinical Anxiety Scale (CAS), Disability Rating Scale (DRS), and higher scores on the Satisfaction With Life Scale (SWLS), Mini Mental State Examination (MMSE) and SF-36 Quality of Life Scale (QOL).

**STUDY PROCEDURE**

**Subject recruitment**

Subjects were recruited from the Brain injury clinic at Johns Hopkins Bay view Medical Center, other Johns Hopkins outpatient clinics, and via advertisements in local papers.

**Inclusion criteria**

(1) Closed head injury, defined as externally inflicted trauma without skull fracture. (2) Presence of DSM IV criteria for ‘Major Depressive Disorder’; (3) ≥18 years of age; (4) Ability to provide informed consent; and, (5) Stable medical history defined as absence of medical problems that requires immediate treatment from physicians and/or hospitalization.

**Exclusion criteria**

(1) History of Stroke, encephalitis, seizures or any other pre-TBI neurological diseases; (2) History of Mental Retardation; (3) Alcohol/substance dependence within the last year, as diagnosed by Structured Clinical Interview for Axis I DSM-IV Disorders (SCID); (4) Subjects with contraindication to MRI scan; (5) Pregnancy (6) Current use of any psychotropics except for trazodone and lorazepam; (7) Poor response to escitalopram in the past; (8) Acute suicidality or requirement for inpatient psychiatric hospitalization, as determined by the study psychiatrist; and, (9) Good response to another antidepressant in the past.

**Assessment measures**

The Structured Clinical Interview for Axis I DSM-IV Disorders (SCID) [21] was used to establish the diagnosis of major depressive disorder and/or other Axis I psychiatric disorders. The SCID-IV is administered by a clinician and includes an introductory overview followed by nine modules, seven of which represent major Axis-I diagnostic classes. Using a decision tree approach, the SCID guides the clinician in testing diagnostic hypotheses as the interview is conducted. The output of the SCID is a record of the presence or absence of each of the disorders being evaluated, for current episode and for lifetime occurrence. It has been found to be diagnostically accurate and significantly better than the unstructured traditional diagnostic assessments.
Primary efficacy measures: Primary outcome measures included scores on the:

Montgomery Depression Rating Scale (MADRS) [22]: The MADRS is an extensively used scale to assess depression. It has been found to exhibit both construct validity, concurrent validity relative to the Hamilton Depression Scale, as well as inter-rater reliability. The scores range from 0-60 with higher scores indicating increased severity of depression. Response was defined as reduction in MADRS scores by 50% compared to baseline. Remission was defined as MADRS scores less than 7 at end of treatment.

Clinical Global Impression (CGI) [23]: CGI-Improvement is a scale used to assess treatment response in psychiatric patients. The 7-item Global Improvement component of the scale will be used to rate how the participant’s illness has improved or worsened compared to baseline (1=very much improved; 7=very much worse). Based on these scores they will be divided into 2 groups: No improvement (Scores 4-7); Yes improvement (scores 3, 2 and 1).

Secondary Efficacy Measures: Secondary outcome measures included scores on the:

Clinical Anxiety Scale (CAS) [24]: The CAS is an instrument derived from the Hamilton Anxiety Rating Scale. It has found to be reliable and valid in the assessment of anxiety and is also effective to evaluate the effects of treatment interventions.

Mini Mental State examination [25] (MMSE): The MMSE is a 30 item bedside measure of cognitive impairment.

SF -36 Quality of life scale (QOL) [26]: The SF-36 QOL is used to assess quality of life. It is a short-form health questionnaire and assesses both physical and mental health. It has been widely used in both the general population and in specific populations with chronic medical illness.

Satisfaction with Life (Satisfaction with Life Scale – SWLS) [27]. The SWLS is a global measure of life satisfaction. It consists of 5-items that are completed by the individual whose life satisfaction is being measured.

Disability Rating Scale (DRS) [28]: The DRS is a measure of impairment, disability and handicap. It is intended to measure accurately general functional changes over the course of recovery and has found to be both valid and reliable. Medical co-morbidity; The General medical health rating (GMHR) [29]. The GMHR is a 4-point clinician administered, global rating scale based on the patient’s medical history and current medications. The scale is designed to quantify in a single number the severity of the patient’s medical problems. The numbers range from 4 which is excellent medical health with no unstable medical conditions and absent or very few medications to 1 which is poor with several unstable medical conditions and several mediations.

Randomization and treatment

Randomization & Treatment: After written informed consent was obtained from all subjects, the study pharmacist randomized the subjects to escitalopram or placebo treatment using a uniform distribution in 1:1 ratio. Treatment with escitalopram was started at 10 mg, and the dose was gradually increased after 4 weeks to 20 mg, if deemed appropriate by the study psychiatrist. No further medication adjustments were made after 8 weeks. Treatment was provided in the form of a single pill to be taken in the morning.

The placebo group received a placebo which appeared similar to the 10 and 20 mg escitalopram.

Study participants were allowed to continue medications necessary to treat any co-morbid medical disorders. Concomitant treatment with other psychiatric medications was not permitted. The exception was use of trazodone up to 75 mg at bedtime as a sleep aid, and up to three 0.5 or 1 mg doses of lorazepam for acute agitation per week during the course of the study and/or prior to the brain scan.

Proton Magnetic Resonance Spectroscopy (MRS)

Proton Magnetic Resonance Spectroscopy (MRS): Proton MRS was performed at 3T (Philips Medical Systems). Multi slice magnetic resonance spectroscopic imaging (MRSI) was performed with repetition time (TR) of 1850 ms, echo time (TE) of 140 ms, and 15 mm slice thickness, in 3 supra-tentorial slices covering the basal ganglia, thalamus, and frontal regions. Single voxel spectroscopy (using the PRESS technique with TR/TE=1700/37 ms, 128 repetitions, and voxel size of 25 x 15 x 15 mm3) was performed bilaterally in the hippocampus. The scan time was 4 minutes 10 seconds for each single voxel and 7 minutes 30 seconds for MRSI. Single-voxel spectroscopy was used for the hippocampus to maximize the quality of the data and to avoid susceptibility artifacts.

In addition, the magnetic resonance imaging protocol also included T1 and T2 weighted sequences: T2-weighted-Fluid-Attenuated Inversion Recovery (FLAIR), three-dimension (3D) magnetization-prepared rapid gradient-echo (MP-RAGE) and Susceptibility weighted imaging (SWI). The total scan time was 35 minutes.

Regions of Interest: The brain regions of interest for this study included bilateral frontal cortical, basal ganglia, thalamus and hippocampal regions.

Statistical analyses included between-group comparisons of data at baseline by the Fisher’s exact test for categorical variables and ANOVA for continuous variables. Linear regression models were used for comparing changes in outcomes between treatment groups at baseline verses end of treatment. Random-effects models for repeated measures with 4 time points were conducted to assess within- and between-group differences over time between the two groups since there were a number of missing values in each outcome. Differences in brain metabolite ratios between the two groups were assessed by subtracting post-treatment ratios from pre-treatment ratios (i.e end of treatment values subtracted from baseline values) and then comparing the differences between the groups by Wilcoxon rank-sum test.

RESULTS

Number of subjects

A total of 132 subjects were screened. Only 16 met the inclusion and exclusion criteria and agreed to participate.
Common reasons for screen failures included: history of substance abuse or substance dependence, concerns about being on placebo, history of TBI prior to age 18; history of seizures, open head injuries; and inability to attend clinic visits.

Of the 16 who consented one failed to return for any follow-up visits and another admitted to active alcohol abuse before initiating medications, and therefore no longer met inclusion criteria. Of the 14 subjects who began treatment, I withdrew from the study secondary to substance use-related legal problems. However, this participant had one follow-up visit and therefore his MRS and neuropsychiatric and neuropsychological data were included in the analyses. No participants withdrew because of side effects.

### Comparison of demographic variables

There were no significant differences between the two groups on any of the demographic variables (Table 1). Motor vehicle accident was the most common cause of TBI in both groups (50% in the escitalopram group and 66.7% in the placebo group). Falls accounted for 12.5% in the escitalopram group versus 33.3% in the placebo group. Assaults were 37.5% in the escitalopram group and 0% in the placebo group. There were no differences between the two groups on injury severity; Fifty percent of subjects in the placebo group and 37.5% in the escitalopram group had mild TBI (p-value = 1.00). Both groups had a long duration since injury (79.4 months versus 101.2 months, p-value = 0.69). There were no differences between the two groups on medical co-morbidity, as assessed by the General Medical Health Rating Scale.

No differences were also noted in the duration of depression between the escitalopram group and the placebo group (10 months versus 33.2 months respectively; p-value = 0.21). Both groups had high rates of pre-TBI psychiatric illness (37.5% in the escitalopram group vs 50% in the placebo group; p-value = 1.00). Thirty percent of patients in the placebo group had pre-TBI depression compared to 25% among escitalopram group (p-value = 1.00). The escitalopram group compared to the placebo group (100% vs 33.3%; p-value = 0.02) had higher comorbid anxiety disorder at the time of the initial evaluation as assessed by the SCID. However, scores on the CAS only showed a trend towards statistical significance (14.8% vs 9.6%; p-value = 0.13). No difference between two groups was observed on comorbid aggression, alcohol or substance abuse.

### Comparison of Outcome Measures (Table 2)

**Primary Outcome Measures:** Both groups showed improvement in depressive symptoms as assessed by the MADRS and in global clinical improvement as assessed by the CGI scale. The between group differences were not statistically significant differences.

Rates of response and remission at 12 weeks were 75% and 62.5% in escitalopram group, respectively, and 66.7% and 50% in placebo group, respectively.

### Secondary Outcome Measures

There were no statistically significant differences between the two groups on change in any of the secondary outcomes measures – anxiety, quality of life, satisfaction with life, disability and/or cognition. (Table 2) shows pre and post treatment differences between the two groups on the respective scales (CAS, QOL, SWL and DRS, MMSE)

### Comparison of Side-Effects

In the comparison of side-effects between the two groups, the escitalopram group had higher rates of indigestion compared to the placebo group (14% vs 2%; p-value = 0.01). In addition, there was a trend towards higher rates of decreased libido in the escitalopram (6.3%) compared to the placebo group (0%; p-value = 0.07) and increased anxiety in the escitalopram (19%) compared to the placebo group (8.3%; p-value = 0.09). There were no other statistically significant differences or trends between the two groups. There were also no serious adverse side effects associated with escitalopram treatment.

### MRSI measures

There were no pre-treatment differences between the two groups in the brain metabolites in any of the brain regions. Regarding post- and pre-treatment differences in brain metabolite ratios, there were no statistically significant differences between the two groups on any of the brain metabolite ratios in any of the brain regions assessed (Table 3). However, a trend towards statistical significance (p-value = 0.01) was noted in the right frontal cortex with higher Cho/Cr ratio in escitalopram compared to placebo group after treatment (median values: -0.08 versus 0.13; p-values = 0.08) and in the right thalamus with higher...
Table 2: Comparison of Placebo and Escitalopram groups on Outcome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline Mean (SD)</th>
<th>Escitalopram Mean (SD)</th>
<th>Between group t (P)</th>
<th>Baseline vs. End t (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI</td>
<td>4.5(0.5)</td>
<td>4.8(0.5)</td>
<td>2.7(1.2)</td>
<td>2.1(0.6)</td>
</tr>
<tr>
<td>MADRS</td>
<td>29.5(3.9)</td>
<td>33.6(6.9)</td>
<td>11.2(9.5)</td>
<td>7(5.7)</td>
</tr>
<tr>
<td>CAS</td>
<td>9.8(3.8)</td>
<td>14.8(6.7)</td>
<td>6.3(4.3)</td>
<td>3.9(5)</td>
</tr>
<tr>
<td>SWL</td>
<td>13.8(7.7)</td>
<td>15.6(9.3)</td>
<td>21.2(9.2)</td>
<td>22.6(5.7)</td>
</tr>
<tr>
<td>QOL</td>
<td>62.8(22.3)</td>
<td>64.1(24.6)</td>
<td>75.2(30.3)</td>
<td>78.7(17.3)</td>
</tr>
<tr>
<td>DRS</td>
<td>1(1.3)</td>
<td>1.4(1.1)</td>
<td>0.7(1)</td>
<td>0.6(0.3)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.3 (1.6)</td>
<td>27.1 (1.03)</td>
<td>29.7 (0.5)</td>
<td>29.0 (0.6)</td>
</tr>
</tbody>
</table>

Abbreviations: CGI: Clinical Global Impression Scale; MADRS: Montgomery Asberg Depression Rating Scale; CAS: Clinical Anxiety Scale; SWL: Satisfaction With Life; QOL: Quality Of Life; DRS: Disability Rating Scale, MMSE: Mini Mental State Exam

Table 3: Comparison of brain metabolite ratios in different brain regions between placebo and escitalopram groups.

<table>
<thead>
<tr>
<th>Ratios*</th>
<th>Placebo (N=6)</th>
<th>Escitalopram (N=8)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Median (min, max)</td>
<td>N</td>
<td>Median (min, max)</td>
</tr>
<tr>
<td>Left Hippocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA+NAAG/Cr</td>
<td>6 -0.13 (-0.28, 0.12)</td>
<td>7 -0.03 (-0.26, 0.16)</td>
<td>-1.00</td>
</tr>
<tr>
<td>Glu+Gln/Cr</td>
<td>6 -0.16 (-1.66, 0.81)</td>
<td>7 -0.10 (-0.19, 0.61)</td>
<td>-0.57</td>
</tr>
<tr>
<td>Right Hippocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA+NAAG/Cr</td>
<td>6 -0.05 (-0.39, 0.23)</td>
<td>7 -0.01 (-0.37, 0.18)</td>
<td>0.00</td>
</tr>
<tr>
<td>Glu+Gln/Cr</td>
<td>6 0.28 (-0.43, 0.76)</td>
<td>7 0.36 (-0.06, 0.65)</td>
<td>0.00</td>
</tr>
<tr>
<td>Left Putamen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>2 0.14 (0.0, 0.28)</td>
<td>1 0.16 (-)</td>
<td>0.00</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>2 -0.43 (-0.47, -0.39)</td>
<td>1 0.23 (-)</td>
<td>-1.23</td>
</tr>
<tr>
<td>NAA/Cho</td>
<td>2 -0.47 (-0.48, -0.46)</td>
<td>1 -0.10 (-)</td>
<td>-1.23</td>
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<tr>
<td>Right Putamen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>2 0.24 (0.03, 0.45)</td>
<td>2 0.07 (0.03, 0.10)</td>
<td>0.41</td>
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<tr>
<td>NAA/Cr</td>
<td>2 0.40 (-0.09, 0.89)</td>
<td>2 -0.31 (-0.82, 0.21)</td>
<td>0.78</td>
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<tr>
<td>NAA/Cho</td>
<td>2 -0.02 (-0.17, 0.14)</td>
<td>2 -0.49 (-1.03, 0.05)</td>
<td>0.78</td>
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<tr>
<td>Left Thalamus</td>
<td></td>
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<tr>
<td>Cho/Cr ratio</td>
<td>5 0.10 (-0.52, 0.25)</td>
<td>4 0.07 (-0.30, 0.27)</td>
<td>-0.25</td>
</tr>
<tr>
<td>NAA/Cr ratio</td>
<td>5 -0.50 (-1.37, 0.24)</td>
<td>4 0.00 (-0.69, 0.71)</td>
<td>-1.23</td>
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<tr>
<td>NAA/Cho</td>
<td>5 0.05 (-2.03, 0.26)</td>
<td>4 -0.05 (-0.53, 0.30)</td>
<td>-0.12</td>
</tr>
<tr>
<td>Right Thalamus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho/Cr ratio</td>
<td>4 -0.01 (-0.17, 0.12)</td>
<td>6 -0.08 (-1.05, 0.33)</td>
<td>0.43</td>
</tr>
<tr>
<td>NAA/Cr ratio</td>
<td>4 0.19 (-0.42, 0.61)</td>
<td>5 -0.12 (-1.38, 0.15)</td>
<td>1.72</td>
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<tr>
<td>NAA/Cho</td>
<td>4 0.25 (-0.25, 0.54)</td>
<td>6 0.14 (-0.52, 1.26)</td>
<td>0.21</td>
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<tr>
<td>Left Frontal Cortex</td>
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<tr>
<td>Cho/Cr ratio</td>
<td>4 -0.09 (-0.35, 0.11)</td>
<td>2 0.23 (0.14, 0.32)</td>
<td>-1.85</td>
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<tr>
<td>NAA/Cr ratio</td>
<td>4 -0.38 (-0.85, -0.02)</td>
<td>2 0.28 (0.10, 0.46)</td>
<td>-1.85</td>
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<tr>
<td>NAA/Cho</td>
<td>4 -0.02 (-0.31, 0.53)</td>
<td>2 -0.40 (-0.42, -0.35)</td>
<td>1.85</td>
</tr>
<tr>
<td>Right Frontal Cortex</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cho/Cr ratio</td>
<td>4 0.13 (0.06, 0.15)</td>
<td>3 -0.08 (-0.24, 0.01)</td>
<td>1.78</td>
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<tr>
<td>NAA/Cr ratio</td>
<td>4 0.19 (-0.37, 0.28)</td>
<td>3 -0.44 (-0.62, 0.31)</td>
<td>0.71</td>
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<tr>
<td>Region</td>
<td>NAA/Cho ratio</td>
<td>Cho/Cr ratio</td>
<td>NAA/Cr ratio</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------</td>
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<td>--------------</td>
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<tr>
<td>NAA/Cho</td>
<td>-0.24 (-0.29, 0.07)</td>
<td>0.78 (0.25, 1.31)</td>
<td>-0.49 (-1.10, 0.12)</td>
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<tr>
<td>Right Prefrontal Cortex</td>
<td>2</td>
<td>1</td>
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<thead>
<tr>
<th>Region</th>
<th>NAA/Cho ratio</th>
<th>Cho/Cr ratio</th>
<th>NAA/Cr ratio</th>
<th>NAA/Cho</th>
<th>Left Frontal White Matter</th>
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<tbody>
<tr>
<td>NAA/Cho</td>
<td>0.12 (-0.14, 0.32)</td>
<td>0.08 (0.06, 0.10)</td>
<td>-0.40 (-0.74, -0.06)</td>
<td>2</td>
<td>0.11 (0.01, 0.21)</td>
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<tr>
<td>Right Frontal White Matter</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0.01 (-) 0.45 0.655</td>
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<table>
<thead>
<tr>
<th>Region</th>
<th>NAA/Cho ratio</th>
<th>Cho/Cr ratio</th>
<th>NAA/Cr ratio</th>
<th>NAA/Cho</th>
<th>Left Corona Radiata</th>
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<tbody>
<tr>
<td>NAA/Cho</td>
<td>0.12 (-0.34, 0.36)</td>
<td>0.99 (0.41, 1.04)</td>
<td>-0.74 (-0.96, 0.70)</td>
<td>3</td>
<td>0.01 (-0.25, 0.41)</td>
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<tr>
<td>Right Corona Radiata</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>-0.42 (-) 1.34 0.180</td>
</tr>
</tbody>
</table>

Changes were also noted in the left frontal cortex with higher NAA/Cho ratio (median values: -0.40 versus -0.02; p-value = 0.06), lower NAA/Cr ratio (median values: 0.28 versus -0.38; p-value = 0.06) and lower Cho/Cr ratio (median values: 0.23 versus -0.09; p-value = 0.06) in escitalopram compared to placebo group after treatment.

**DISCUSSION**

There were no statistically significant differences between the group treated with escitalopram versus the group treated with placebo on either the primary or secondary outcome clinical variables. Similarly there were no differences between the two groups on brain metabolite ratios pre versus post-treatment.

The lack of statistically significant differences in the two groups could be due to the treatment chosen, as ( escitalopram) may not be effective for the treatment of TBI depression. A second factor could be the complex pathology of TBI depression. It is possible that similar to other neurodegenerative disorders, TBI depression has a more complex pathology than idiopathic depression in the absence of a neurodegenerative disorder. Poor response to serotonergic agents was also found in depression associated with Alzheimer’s disease [30]. It may be that the nature of the serotonergic deficit in these conditions are different from primary major depression or that other neuropathology or neuro chemical deficits are involved. A third factor could be the study sample, including the small number of patients, long duration since TBI, and varying duration of depression. Having a more homogenous sample with regards to the severity of TBI, a shorter duration of time since TBI and larger sample size may have produced different results and enabled us to answer the question of whether or not escitalopram is effective for treatment of TBI depression. Finally, medical issues may have been a factor, including the presence of medical problems, continuation of medications to treat comorbid medical conditions and intermittent use of sleep aids such as lorazepam or trazodone. These variables may also have influenced the observed response to treatment and MRSI findings. Patient enrollment was also a challenge with many patients failing the screening evaluation. Recruitment may have been more successful if we were able to revise our study criteria to only exclude subjects with history of active substance misuse in the last month, (instead of the 1-year history of substance abuse and or dependence), conduct home visits, and consider a study design in which all patients can be exposed to both the placebo and the active agent, such as placebo-run in trial, or sequential parallel comparison design trial. We were also unable to obtain good quality MRSI data in all our participants. This is another limitation of the study and may lead to a lack of statistical power.
partially explain the lack of differences between the two groups. The reasons for poor quality MRSI data include motion artifacts in some subjects and susceptibility artifacts affecting data quality in the frontal regions. In the future, minimizing length of the protocol, possibly scheduling two sessions for structural Magnetic Resonance Imaging and MRSI and for single voxel MRS would be helpful in improving data quality.

Despite these limitations, this is the only study to date to assess neuroimaging changes of treatment response in subjects with TBI depression. Even though there were no statistically significant differences between the two groups on brain metabolite ratios post-treatment, a trend towards statistical significance was noted in the left frontal region. However, the results are inconsistent as both high NAA/Cho and low NAA/Cr ratios were noted. The escitalopram group also had higher NAA/Cr in the right thalamus, which also trended toward statistical significance.

Thalamic dysfunction has been noted in both major depression and TBI depression. In a small case-control pilot study comparing subjects with TBI depression to age matched non-TBI non-depressed controls, Rao et al [31] found reduced NAA/Cho in the thalamus and reduced NAA/Cr in the basal ganglia in patients with TBI depression. There are no studies that have looked at NAA+NAAG/Cr or Glu/Gln/Cr ratios in TBI depression. However, in their review on MRS studies of glutamate-related abnormalities in mood disorders, Yüksel and Öngür note that there is ‘suggestive evidence for reduced glutamine/glutamate ratio’ in studies of depression [13]. Pfeiderer et al and Michael et al also found normalization of levels of glutamate metabolites in the prefrontal cortex after electroconvulsive treatment [32,33].

There are several studies using MRSI and PET methods in idiopathic major depressive disorder indicating that effective treatment with SSRIs is associated with increased glucose metabolism and neurochemical changes in the anterior cingulate cortex and medial prefrontal cortex [34-36]. Studies in idiopathic major depression have also noted an association between major depression and hyper-activity in the thalamus [37-39]. Young et al [40] conducted a neuro anatomical postmortem tissue study to determine relationships between thalamic volume and major depressive disorder and the 5HTTLPR genotype. They found that major depression, the short allele of the serotonin transporter gene (5-HTTLPR) and suicide were independent factors associated with thalamic enlargement. Major depression was specifically associated with enlargement of the limbic nuclei in the thalamus and antidepressant treatment was associated with reduced thalamic volume. Charles et al [41] have also shown increased NAA/Cho levels in both gray and white matter at the level of the third ventricle (including basal ganglia and thalamus) in seven subjects with major depression compared to age matched controls after treatment with nefazodone.

A PET study reported progressive alterations in glucose metabolism after 8-10 weeks after citalopram treatment in geriatric depressed patients relative to comparison subjects [42]. After a single, intravenous dose of citalopram, decreased metabolism in cortico-cortical circuits was observed in geriatric depressed patients, specifically in the left middle frontal and inferior parietal cortices. After 8 weeks of treatment with citalopram, increases in the right putamen, bilateral occipital cortex, and left cerebellum were observed [42]. In summary, even though we did not find significant clinical or metabolic changes in patients treated with escitalopram compared to placebo in our pilot study, there is evidence in the literature from non-TBI studies that antidepressant treatment is associated with clinical improvement and biochemical changes in the brain. Findings from this study underscore the importance of gaining a better understanding of the neurobiology of TBI depression, so that more effective treatments can be developed. MRSI and other neuroimaging studies may be helpful not only in elucidating the neurobiology of TBI depression, but also have the potential to provide biomarkers for illness and treatment response.

**CONCLUSION**

Neuroimaging studies performed during clinical trials are important as they have the potential to increase the understanding of the neurobiological mechanisms of TBI-related depression, and could provide objective markers of the impact of antidepressant treatment. Our pilot study reveals preliminary evidence of subtle changes in brain metabolites in the frontal regions and the thalamus. Lessons learned from this pilot study can be used to design larger studies that have the potential to answer the question on the nature of brain metabolite changes associated with response to treatment of TBI depression.

**REFERENCES**

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