Pediatric Demyelinating Diseases of the Central Nervous System, Imaging and Clinical Review

Senior Thesis

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In partial fulfillment of the requirements for the degree of Bachelor of Science

by
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<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>ADEM</td>
<td>Acute Disseminated Encephalomyelitis</td>
</tr>
<tr>
<td>ASIA</td>
<td>American Spinal Injury Association</td>
</tr>
<tr>
<td>ATM</td>
<td>Acute Transverse Myelitis</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CIS</td>
<td>Clinically Isolated Syndrome</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
</tr>
<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
</tr>
<tr>
<td>GM</td>
<td>Gray Matter</td>
</tr>
<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LMN</td>
<td>Lower Motor Neuron</td>
</tr>
<tr>
<td>M₀</td>
<td>Signal intensity without saturation pulse</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>Mₜ₀sat</td>
<td>Signal intensity with saturation pulse</td>
</tr>
<tr>
<td>MT</td>
<td>Magnetization Transfer</td>
</tr>
<tr>
<td>MTI</td>
<td>Magnetization Transfer Imaging</td>
</tr>
<tr>
<td>MTR</td>
<td>Magnetization Transfer Ratio</td>
</tr>
<tr>
<td>ON</td>
<td>Optic Neuritis</td>
</tr>
<tr>
<td>PNS</td>
<td>Peripheral Nervous System</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>RRMS</td>
<td>Relapse-Remitting Multiple Sclerosis</td>
</tr>
<tr>
<td>SCI</td>
<td>Spinal Cord Injury</td>
</tr>
<tr>
<td>TM</td>
<td>Transverse Myelitis</td>
</tr>
<tr>
<td>WM</td>
<td>White Matter</td>
</tr>
</tbody>
</table>
Abstract

A limited amount of research has explored the relationship between clinical status and lesion characteristics in pediatric patients with demyelinating diseases. Although the presence of a demyelinating lesion can be confirmed through Magnetic Resonance Imaging (MRI), studies have shown MRI to have poor correlation with clinical status. However, recently the use of advanced imaging techniques such as Magnetization Transfer Imaging (MTI) has helped to bridge the gap by exploiting demyelination. This study is the first to evaluate the relationship between MTI analysis of the spinal cord and clinical status in pediatric patients with myelitis. An acute TM patient and a sub-acute MS patient were compared to a control group (n=3). The mean MTR values and mean cross-sectional area were lower in the patients compared to the controls. Spearman’s rank correlation analyses revealed a strong, negative correlation between MTR values and motor symptoms ($r=-0.87$, $p=0.03$) and MTR values and pain symptoms ($r=-0.87$, $p=0.03$). Interestingly, the MTR analysis was more sensitive at the level of injury in the TM patient, while the cross-sectional area analysis was more sensitive in the MS patient. Although the relatively small sample size limits the reliability of the results, the reduced MTR values in patients compared to controls suggests the reproducibility of MTI analysis. With more patients, a future study could validate the feasibility of this technique for clinical use, which will help to improve the diagnosis and treatment for pediatric patients with demyelinating diseases.
Introduction

Spinal Cord

As part of the Central Nervous System (CNS), the spinal cord serves as the main bridge between the brain and the rest of the body. Longitudinally divided into 5 distinct regions, the spinal cord spans from the brainstem to the lumbar region and is composed of 31 segments (see Figure 1A)\(^1,3\). The spinal cord is organized into columns of gray matter (GM) and surrounding white matter (WM). From a cross-sectional view, the GM appears in a butterfly shape. Each level of the spinal cord has a slightly different distribution of GM and WM, descending from the brain the amount of WM decreases (see Figure 1B)\(^2\).

![Figure 1. Spinal Cord. (A) The five regions of the spinal cord are labeled from top to bottom: Cervical (blue), Thoracic (green), Lumbar (yellow), Sacral (red), and Coccygeal (gray). Adapted Image\(^1\). (B) Cross-sections of the cervical spinal cord including C1, C2, and C5. This depiction of the cross-sections of the spinal cord at different vertebral levels illustrates the difference in the distribution of GM and WM along the spinal cord. Adapted Image\(^2\).]
The “wings” of the butterfly are termed horns and divided into a dorsal horn and a ventral horn. Each segment of the spinal cord connects to a ventral root, part of the motor system, and a dorsal root, part of the sensory system, which combine to form spinal nerves on either side of the spinal cord. Figure 2 illustrates the dorsal column, shaded in blue, and the ventral column, shaded in red, which are part of the WM in the spinal cord.

Thus, the neurons within the spinal cord are responsible for relaying information about sensation and outputting motor commands. Therefore, the spinal cord serves as the pivotal link connecting the CNS to the Peripheral Nervous System (PNS). The dorsal, or posterior, horn receives signals from the dorsal root ganglion, conveying information about the sensory system into the CNS. This ascending tract sends signals to the thalamus by way of the lateral spinothalamic
tract. In a laminar configuration, fibers that reach the thalamus convey information about pain and temperature sensation to the brain. The motor neuronal pathway is composed of axons projecting along the corticospinal tract starting in the primary motor cortex and follows a series of paths to eventually terminate in the GM of the spinal cord. Motor axons exit via the ventral, or anterior, horn of the spinal cord. This descending pathway affects the activity of both alpha and gamma lower motor neurons (LMN), which influences limb and posture control.

Demyelination

Myelin is a fatty substance that surrounds the axon of a neuron. The myelin sheath serves as an insulator that wraps around the neuron and increases the speed of propagation in cell to cell signaling. Tissue in the CNS can be classified based on the presence or absence of myelin. WM appears in nerve tissues that have myelin due to its white appearance, whereas GM signifies the lack of myelin surrounding an axon in a bundle of nerve cells.

![Figure 3. Neuron with myelin and damaged myelin. The image depicts a neuron with each of its parts labeled. The two boxes above the neuron illustrate an axon with either healthy myelin or damaged myelin. Demyelination leads to the destruction and loss of the myelin sheath.](image)
In certain diseases, myelin can become damaged, which is referred to as demyelination or “a pathological process of destruction of myelin-supporting cells” \(^9\). A pictorial representation of a neuron with myelin loss can be seen in Figure 3. Ultimately, demyelination leads to a loss of myelin, resulting in decreased speed of action potential conduction between neurons in the CNS \(^10\). In response to the damaged tissues in the CNS, the body’s immune system treats the injury much as it would an inflammatory condition, resulting in a neuro-inflammatory response. Demyelinating diseases of the CNS can affect several areas, including the optic nerve(s), spinal cord, cerebrum, brainstem, and cerebellar regions \(^11\). A broad range of diagnoses exist in order to properly classify demyelinating diseases based on the location of a patient’s injury or injuries. Characteristics of lesions affecting nerves and roots of each vertebral level of the cervical spinal cord and its corresponding clinical outcome can be found in Table 1 \(^4\).

### Table 1. Lesions affecting the cervical spinal cord

<table>
<thead>
<tr>
<th>Lesion Affecting</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Minor motor difficulties</td>
</tr>
<tr>
<td>C2</td>
<td>Sensory disturbances; motor difficulties of the head and neck</td>
</tr>
<tr>
<td>C3</td>
<td>Sensory disturbances of the upper neck; paresis of the neck, shoulder, and diaphragm</td>
</tr>
<tr>
<td>C4</td>
<td>Sensory disturbances occur at the lower neck; paresis of the neck</td>
</tr>
<tr>
<td>C5</td>
<td>Pain in the neck, shoulder, and upper arm; sensory disturbances of the arm; paresis</td>
</tr>
<tr>
<td>C6</td>
<td>Pain in the arm; sensory disturbances of the arm and hand; paresis</td>
</tr>
<tr>
<td>C7</td>
<td>Pain in the arm; sensory disturbances of the hand; paresis; pseudomyotonia</td>
</tr>
<tr>
<td>T1</td>
<td>Sensory disturbances of the arm; paresis</td>
</tr>
</tbody>
</table>

### Multiple Sclerosis

Multiple Sclerosis (MS) is defined as “a disease of the CNS characterized by the loss of motor and sensory function, resulting from immune-mediated inflammation, demyelination, and
subsequent axonal damage” 12. Approximately, 3.6/100,000 adult cases of MS have been reported per year 13, whereas between 0.18 and 0.51/100,000 pediatric cases of MS have been estimated to occur yearly 11. The different clinical courses associated with MS include: clinically isolated syndrome (CIS), relapse-remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS) 14. Pediatric MS patients primarily experience RRMS with a higher relapse rate compared to adult MS patients 15. One requirement for a diagnosis of MS is dissemination of disease activity within the CNS 16. An MS diagnosis is based on the revised McDonald criteria by the International Panel on the Diagnosis of Multiple Sclerosis in 2010 17,18.

Transverse Myelitis

Transverse Myelitis (TM) is characterized as a neurological disorder manifested by inflammation of the spinal cord. By definition ‘myelitis’ means inflammation of the spinal cord and ‘transverse’ refers to the specific location of the spinal cord that has been inflamed 19. According to the National Institute of Neurological Disorders and Stroke (NINDS), the inflammation must be present across both sides of one segment of the spinal cord 20. Acute Transverse Myelitis (ATM) can occur in two different forms- either as an isolated inflammatory disorder or as part of a multifocal CNS demyelinating disease 19,21. Approximately, 1 to 8 cases per million people in the United States report incidence of ATM with 1400 newly diagnosed cases per year in the United States 22. An estimated 20% of the cases reported occur in patients less than 18 years of age 21,23.

Clinical Status

A multitude of symptoms arise in patients who suffer from MS and TM. A broad generalization of the symptoms that occur in both diseases include motor, sensory, and autonomic dysfunction (see Table 2). More specifically, the most common symptoms at onset for patients with TM
include: sensory loss or numbness (91%), weakness (89%), urinary dysfunction (85%), and pain (75%) 
While, pediatric MS patients experience several of the same symptoms, these patients also present with seizures and 30-40% have cognitive dysfunction. It is well known that damage to the CNS commonly induces neuropathic pain. The International Association for the Study of Pain (IASP) defines neuropathic pain as pain arising from a lesion or disease to the somatosensory nervous system. Clinically, symptoms are divided into painful (i.e. allodynia and hyperalgesia) and non-painful (i.e. paresthesia and dysesthesia) classifications. It has been reported that approximately 60% of pediatric TM patients experience pain as an initial symptom. Another study found that out of 24 children, 88% reported feeling pain. Approximately, 75% of adult MS patients suffer from painful symptoms. Regardless of these findings, very little is known about pain in pediatric patients who suffer from demyelinating diseases. Overall, TM and MS patients experience a wide array of symptoms. Following a diagnosis, a management team works with the patient to provide the appropriate treatment and supportive services.

**Magnetic Resonance Imaging**

Conventional Magnetic Resonance Imaging (MRI) protocols have served as a diagnostic and monitoring tool for clinicians in the assessment of MS and TM. Table 2 outlines the specific criteria for the clinical presentation and imaging manifestations of MS and TM. MRI is a non-invasive imaging technique that is currently the primary method used to check for the involvement of inflammation in patients diagnosed with a demyelinating disease. Using MRI contrast enhancement agents, primarily gadolinium-based, has become a standard practice because the contrast agents allow for better detection of lesions. The human body is predominantly composed of water and contains millions of hydrogen protons associated with larger macromolecules. In the presence of a strong magnetic field, the protons will align in part to that
field. When a radiofrequency (RF) is applied the protons become excited and spin, stopping the RF field leads to a release in energy by the protons to realign with the magnetic field\textsuperscript{37}. MRI helps detect both anatomical and pathological changes in tissue, and the changes in tissue can be exploited by altering the contrast as either a T\textsubscript{1} or T\textsubscript{2}-weighted image. Mobile protons have relatively long T\textsubscript{2} relaxation times; in contrast, protons associated with tissues in the body that have larger macromolecules are characterized by short T\textsubscript{2} relaxation times, which are too short to be detected by an MRI scanner\textsuperscript{38,39}. Therefore, the shortcoming of a conventional MRI is that it lacks the sensitivity to detect demyelination and axonal loss in tissues with large macromolecules (i.e. the spinal cord)\textsuperscript{40}. As a result, studies have shown that MRI modestly correlates with clinical status, as seen in MS patients\textsuperscript{41,42}.

**Table 2. Criteria for MS and TM diagnosis**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical Presentation</th>
<th>Imaging</th>
<th>Diagnostic Tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>Autonomic Dysfunction, Motor Dysfunction, Sensory Dysfunction, Numbness, Spasticity, Weakness, Fatigue, Pain, Seizures, Cognitive Dysfunction, Vision Problems</td>
<td>• Damage occurred in at least two separate areas of the CNS (brain, spinal cord, or optic nerve) • Damage occurred at least one month apart</td>
<td>• MRI • CSF • Evoked Potentials</td>
</tr>
<tr>
<td>TM</td>
<td>Autonomic Dysfunction, Motor Dysfunction, Sensory Dysfunction Muscle Weakness, Paralysis, Pain, Paresthesia, Fatigue</td>
<td>• Damage to the spinal cord</td>
<td>• MRI • Lumbar Puncture</td>
</tr>
</tbody>
</table>

**Magnetization Transfer Imaging**

Magnetization Transfer Imaging (MTI) is an MRI-based technique reliant on the interaction of energy exchange between protons in free liquid and protons that are bound to macromolecules. In this approach, a resonance radiofrequency pulse is applied to partially saturate the magnetization
of the bound protons, which excites these protons and leads to the manipulation of the free proton pool \(^{38,43}\). When a magnetization or energy exchange is detected in tissues between the partially saturated bound proton pool and the free proton pool, the result is a decreased signal intensity. This decreased signal intensity is greater in WM than GM, in which the WM has been thought to mirror myelination \(^{44}\). **Figure 4** depicts the energy exchange between the free water pool and the bound water pool \(^{45}\). MTI is known to be sensitive to changes in myelin and is used to detect demyelination \(^{44}\). Magnetization Transfer Ratio (MTR) is the output value calculated that signifies the efficiency of the exchange that is generated. This value is derived by computing the difference between the signal intensity without a saturation pulse and the signal intensity with a saturation pulse \(^{46}\). In effect, MTI has an enhanced pathological specificity to certain substrates that a conventional MRI may not be able to detect \(^{44}\).

![Figure 4. Magnetization Transfer](image)

*Figure 4. Magnetization Transfer. The image shows a free water pool and a bound water pool. The bound water is composed of a surface layer of water that is associated with macromolecules. An RF pulse is applied, which results in a transfer of energy between the two proton pools.*

**Literature Review**

Nevertheless, researchers have just started to evaluate MTI techniques as an analytic tool. Therefore, these techniques have not yet been implemented into a clinical setting \(^{39}\). Thus far,
studies have primarily used MTI analysis to assess demyelination of the brain and spinal cord in patients with MS \(^{46-50}\) and patients with spinal cord injuries (SCI) \(^{51}\). A comparison of the previous studies can be found in Table 3 \(^{47,49,51-54}\). Notably, most of the preceding research has examined adult spinal cords. In postmortem MS patients, MTR values have been shown to correlate with myelin content and axonal density \(^{40}\). Additionally, previous studies have identified that MS patients have decreased MTR values in the cervical spinal cord compared to healthy controls \(^{48}\). Lesion anatomical localization has been shown to correlate with functional deficits. Previous research has identified a correlation between Expanded Disability Status Scale (EDSS) scores and MTR values in the spinal cord of patients with MS \(^{49}\). A more recent study assessed the relationship between MTR measures and clinical status in adult patients with spinal cord injuries. The researchers found that MTR values in the dorsal spinal cord predicted sensory deficits, while MTR values in the ventrolateral spinal cord predicted motor deficits \(^{51}\).

**Table 3.** MTI analysis and clinical correlation in spinal cord disease

<table>
<thead>
<tr>
<th>References</th>
<th>Sample Size</th>
<th>Mean age (years)</th>
<th>Diagnosis</th>
<th>Imaged levels</th>
<th>MTR (mean)</th>
<th>Clinical Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolins et al. 2017</td>
<td>N=3</td>
<td>7.86</td>
<td>TM</td>
<td>Thoracic</td>
<td>38.65</td>
<td>PainDETECT</td>
</tr>
<tr>
<td>Lema et al. 2016</td>
<td>N=52</td>
<td>38</td>
<td>MS</td>
<td>Cervical</td>
<td>45</td>
<td>EDSS</td>
</tr>
<tr>
<td>Cohen-Adad et al. 2011</td>
<td>N=14</td>
<td>45</td>
<td>SCI</td>
<td>Cervical</td>
<td>26.5</td>
<td>ASIA</td>
</tr>
<tr>
<td>Filippi et al. 2000</td>
<td>N=96</td>
<td>37.7</td>
<td>MS</td>
<td>Cervical</td>
<td>44.3</td>
<td>EDSS</td>
</tr>
<tr>
<td>Lycklama á Nijeholt et al. 2000</td>
<td>N=65</td>
<td>45</td>
<td>MS</td>
<td>Cervical</td>
<td>30</td>
<td>EDSS</td>
</tr>
<tr>
<td>Silver et al. 1997</td>
<td>N=12</td>
<td>34.7</td>
<td>MS</td>
<td>Cervical</td>
<td>17.95</td>
<td>EDSS</td>
</tr>
</tbody>
</table>
Overall, the previous research has revealed the lack of MTI analysis in pediatric patients with myelitis. Likewise, the relationship between demyelinating lesions and neurological symptoms has never been evaluated in children.

Therefore, the goal of this study was to explore the relationship between MTR values, cross-sectional area, and clinical status of the cervical spinal cord in pediatric patients with demyelinating diseases. It was hypothesized that there would be a relationship between MTR values and clinical status. Another goal of this study was to examine the feasibility of MTI as a tool for clinical analysis.

**Materials and Methods**

**Subjects**

Two patients with cervical spinal cord lesions were recruited. One had acute TM (male, age=13.33 years), and the other had sub-acute MS (male, age=10.92 years). Both patients had cervical spinal cord lesions covering C2 to C4 levels. See Table 4 for more information on disease characteristics and symptoms of both patients. Information regarding patient’s sensory, motor, and pain symptoms were collected from neurology notes located in patients’ medical records. Medical records were accessed through PowerCharts at Boston Children’s Hospital. Symptoms were obtained from reports in the patients’ record that were closest to the date of the MRI scan, to ensure consistency between symptoms and lesion characteristics. Three healthy control patients were recruited (female, age=14.36 ± 2.81 years). The Institutional Review Board approved all experimental protocol for this study, and all participants signed an informed consent waiver before being scanned. This study focused solely on myelitis- classified as a neurological disorder caused by inflammation of the spinal cord. Specifically, the cervical spinal cord was examined, spanning levels C1 to T1.
Table 4. Disease characteristics and symptoms for both patients

<table>
<thead>
<tr>
<th>Disease Characteristics</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>TM</td>
<td>MS</td>
</tr>
<tr>
<td>Type</td>
<td>Acute</td>
<td>Sub-Acute</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.33</td>
<td>10.92</td>
</tr>
<tr>
<td>Onset (days)</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Lesion (spinal cord levels)</td>
<td>C2-C4</td>
<td>C4</td>
</tr>
</tbody>
</table>

**Symptoms**

<table>
<thead>
<tr>
<th>Sensory Changes (R hand)</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor</td>
<td>Gait Changes</td>
</tr>
<tr>
<td>Pain</td>
<td>Neck Pain</td>
</tr>
<tr>
<td>Other</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>L&gt;R Optic Neuritis</td>
</tr>
</tbody>
</table>

**Case Report-TM Patient**

A thirteen-year old male was healthy until one evening after an uneventful soccer practice in October, he began to experience acute neck pain. That evening he had onset of sensory changes, which included distal to proximal paresthesia and bilateral numbness of the upper extremities. When he awoke in the morning, he had difficulty walking due to muscle weakness. This led to a visit to the hospital, where the patient was diagnosed with TM. His level of injury extended from C2 to C4 (see Figure 5). He was then treated with five days of high-dose IV methylprednisolone. He was also treated with gabapentin for pain. After a few weeks, the patient’s symptoms improved; however, he continued to experience sensory changes in his right hand, and had some difficulty with running. In terms of etiology, the patient’s history was significant for having received an influenza vaccination four weeks prior to onset of symptoms. There was no family history of autoimmune disease. Other remarkable information about the patient was a low serum 25-Hydroxyvitamin D concentration of 23.2 ng/mL.
An eleven-year old male presented with three weeks of blurry vision prior to admission. The patient had been seen by an ophthalmologist, but was referred for further evaluation. An MRI scan was obtained of the patient’s brain and spine; multiple T2 lesions consistent with demyelination were identified, particularly at the C4 vertebral level in the spinal cord (see Figure 6). Other symptoms experienced by the patient included, left worse than right optic neuritis (ON), left bicep and deltoid weakness, difficulty with tandem, neck pain, and a headache. Subsequently after this diagnosis, the patient was treated with five days of IV Solumedrol followed by plasma exchange. The patient’s clinical status improved, and he was discharged on Copaxone. Other notable information in regards to the patient was that he had a BMI of 26.1 kg/m² and a low serum 25-
Hydroxyvitamin D concentration of 26 ng/mL. It was noted that the patient had received a flu shot in mid-September.

![Image of MRI scans](https://via.placeholder.com/150)

**Figure 6. MRI of the MS patient.** (A) A sagittal T2-weighted image of the MS patient’s cervical spinal cord. The patient’s injury was at level C4. (B) An axial T2-weighted image at level C4. The arrow points to the region of hyper-intensity.

**Data Acquisition**

Participants were scanned once using a 3T MRI scanner (Siemens Trio). A depiction of the scanner and a T2-weighted image can be seen in Figure 7. Anesthesia, cardiac and/or respiratory gating were not used. If a participant could not tolerate being in the scanner without being sedated, they were excluded from the study. Unenhanced T2-weighted scans of the cervical spine were collected. T1-weighted imaging with and without MT saturation pulses were collected.
Data Processing

Data Pre-Processing

Images were uploaded onto the computer. Subjects’ identities were anonymized to prevent biases based on knowledge of patients and controls. Next, images were assessed for quality assurance. OsiriX, a widely used medical viewer, was used to perform this task. Subjects’ data were imported to OsiriX. The MT<sub>on</sub> axial slices, MT<sub>off</sub> axial slices, and T2-weighted images were selected. Each level and mid-level of the spinal cord (15 axial slices) were ranked either: ok (spine boundaries are somewhat visible, but the distinction between GM and WM are unclear and dorsal and ventral columns are inseparable), good (boundaries are clear between GM and WM and dorsal and ventral columns are separable), poor (boundaries are blurred and the spine is not visible), or indistinguishable (does not fall into one of the previous listed categories). Each ranking was labeled to code each axial slice of the spinal cord. Subjects who had axial slices with primarily good and ok ratings were used in this analysis, while subjects who had axial slices with indistinguishable ratings were removed and not used for further analysis.

Figure 7. MRI scanner and an MRI generated image. Patients undergo an MRI in a Siemens 3T MRI scanner, which can be seen on the left. The black arrow points to the image obtained from the scanner, illustrating part of a patient’s brain and spinal cord Adapted Image 3.6.
**Data Post-Processing**

Magnetization Transfer Imaging was performed using Olea SphereTM (Olea Medical®) 3.0. By using the two images for each axial spinal cord segment, one with a saturation pulse and the other without a saturation pulse. The Olea SphereTM software derived MTR images pixel-by-pixel based the following equation.\(^{38}\)

\[
\text{MTR} = \frac{(M_O - M_{SAT})}{M_O} \times 100
\]

\(M_O\) refers to the signal intensity when there is no saturation pulse for a given pixel, while the \(M_{SAT}\) is the signal intensity when there is a saturation pulse at the same pixel.\(^{57}\) MTI acquisitions were applied in the same anatomic location prescribed by the T2-weighted images. Cervical spinal cord levels C1 to T1 were used. Subjects’ data were imported onto the software, and the \(M_{TO}\) axial slices, \(M_{TSAT}\) axial slices, and T2-weighted images were selected to further process the MTR values.

**ROI-based analysis**

Once images were imported, regions of interest (ROI) were manually drawn on axial slices along the cervical spinal cord using the MTR calculated images. ROIs were drawn based on the anatomical structure of the spinal cord and only included voxels within the spinal cord, excluding cerebrospinal fluid (CSF). An example of an ROI drawn on an axial slice of the spinal cord can be seen in Figure 8. MTR values were collected at each vertebral level of the cervical spinal cord. After each ROI was drawn, a picture was taken to document the slice number and ROI number to ensure that they corresponded with one another. Once all of the MTR values were collected, two patients and three controls were chosen based on two criteria: quality of the images generated from the scanner and age. All of the data for the five subjects were stored in an Excel file. If participants
had upper and lower disc levels covered in their scan, these MTR values were averaged to obtain mid disc spinal cord levels. All participants had 15 axial slices, which were then coded from 0 to 14, where 0 corresponded to the vertebral level C1 and 14 corresponded to the vertebral level T1.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows (v. 24, SPSS Inc., Chicago, IL). Descriptive statistics were collected for MTR values and cross-sectional area, comparing patients to controls. An independent samples T-test was used to assess MTR values and cross-sectional area of each vertebral level of the cervical spinal cord in patient compared to controls. Several correlations were computed using Spearman’s rank correlation coefficient (r). The correlation coefficient is a quantitative value that conveys the strength in association between two variables, ranging from -1 to +1, where -1 and +1 indicate strong relationships and 0 indicates no relationship. A positive correlation coefficient means that
as one variable increases, the other variable increases, or vice versa, as one variable decreases the other variable decreases. A negative correlation coefficient means that one variable increases, while the other variable decreases.

**Results**

**Magnetization Transfer Imaging Analysis**

The MTR value calculated for the entire cervical spinal cord (15 axial slices) of all subjects was found to be 45.34 ± 2.60. A comparison of the mean MTR values of the entire cervical cord between patients and controls revealed the control group to have a mean MTR value of 46.85 ± 0.89, while the mean MTR value for the patients was 43.08 ± 2.91. **Figure 9** graphically compares the MTR values between patients and controls across the entire cervical spinal cord. Whereas, **Figure 10** shows MTR values of the TM patient and MS patient separately in comparison to the control group. Further analysis was done to examine the difference in MTR values at each vertebral level and mid-level (C1-T1). An independent samples T-test was computed separately for the TM patient and the MS patient compared to the control group (see **Table 5**). The TM patient had statistically significant differences in MTR values at six different vertebral levels compared to the control group (p<0.05). Of these six significant regions, three were within the patient’s injury level, C2-C4. The MS patient had statistically significant differences in MTR values at three different vertebral levels compared to the control group (p<0.05). Of these three identified regions, one was at the patient’s level of injury, C4.
Figure 9. MTR values of the cervical spinal cord in patients compared to controls. The blue diamond line represents the control group, while the red square line represents the patients group.

Figure 10. MTR values of cervical spinal cord in each patient compared to controls. The blue triangle line represents the control group, the orange square line represents the MS patient, and the green diamond line represents the TM patient.
Table 5. MTR values of the cervical spinal cord

<table>
<thead>
<tr>
<th>Spinal Cord Levels</th>
<th>Controls</th>
<th>TM Patient</th>
<th>MS Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>51.33</td>
<td>43.98*</td>
<td>48.53*</td>
</tr>
<tr>
<td>C1-C2</td>
<td>48.91</td>
<td>42.21</td>
<td>48.54</td>
</tr>
<tr>
<td>C2-C3</td>
<td>47.34</td>
<td>40.71*</td>
<td>46.46</td>
</tr>
<tr>
<td>C3-C4</td>
<td>47.75</td>
<td>39.24</td>
<td>44.02</td>
</tr>
<tr>
<td>C4-C5</td>
<td>46.14</td>
<td>39.87*</td>
<td>43.81</td>
</tr>
<tr>
<td>C5-C6</td>
<td>46.17</td>
<td>40.33</td>
<td>43.44</td>
</tr>
<tr>
<td>C6-C7</td>
<td>45.03</td>
<td>41.83*</td>
<td>42.69*</td>
</tr>
<tr>
<td>C7-T1</td>
<td>43.06</td>
<td>39.92</td>
<td>49.21</td>
</tr>
</tbody>
</table>

Cross-Sectional Area Analysis

The mean cross-sectional area of the entire cervical spinal cord for all subjects was found to be 49.54 ± 6.99 mm². A comparison of the mean cross-sectional area of the cervical spinal cord between patients and controls revealed the control group to have a mean cross-sectional area of 52.42 ± 7.31 mm², while the mean cross-sectional area for the patient group was 45.20 ± 5.10 mm². Figure 11 compares cross-sectional areas between patients and controls across the entire cervical spinal cord, while Figure 12 shows cross-sectional area between the TM patient, MS patient, and the control group. Further analysis was done to investigate the difference in cross-sectional area at each vertebral level (C1-T1). An independent samples T-test was computed separately for the TM patient and the MS patient compared to the control group (see Table 6). The TM patient was found to have no significant differences in cross-sectional area measures compared to the control group (p>0.05), whereas the MS patient was found to have statistically significant differences.
differences (p<0.05) in cross-sectional area measures at two different vertebral levels compared to the control group. The two levels that were statistically significant were located at levels C4-C5 and mid C5. The MS patient’s injury was located at C4.

![Graph](image1.png)

**Figure 11.** Cross-sectional area of the cervical spinal cord in patients compared to controls. *The blue diamond line represents the control group, while the red square line represents the patients group.*

![Graph](image2.png)

**Figure 12.** Cross-sectional area of cervical spinal cord in each patient compared to controls. *The blue triangle line represents the control group, the orange square line represents the MS patient, and the green diamond line represents the TM patient.*
Correlation Analysis

Several one-tailed, Spearman’s rank correlations were computed to evaluate the relationship between different variables (see Table 7). A Spearman’s rank correlation was used to examine the relationship between MTR values and cross-sectional area at each vertebral level of the spinal cord, (r=−0.13, p=0.13). There was no association between MTR values and cross-sectional area in the cervical spinal cord. Several Spearman’s rank correlations were computed to assess the relationship between mean MTR values and mean cross-sectional area with clinical status (motor, sensory, and pain symptoms). First, a Spearman’s rank correlation was computed to assess the relationship between sensory symptoms and mean MTR values, (r=−0.71, p=0.09) and mean cross-sectional area (r=0.00, p=0.50). There was a weak, negative association between mean MTR values and sensory presentations; however, it was not statistically significant. There was no relationship between sensory symptoms and mean cross-sectional area. Second, a Spearman’s rank correlation was computed to investigate the relationship between motor symptoms and mean MTR values.
values, \( r = -0.87, p = 0.03 \) and cross-sectional area, \( r = -0.58, p = 0.15 \). There was a strong, negative correlation between MTR values and motor symptoms. However, no relationship was found between motor symptoms and cross-sectional area. Third, a Spearman’s rank correlation was evaluated to assess the relationship between MTR values and pain symptoms, \( r = -0.87, p = 0.03 \) and cross-sectional area, \( r = -0.58, p = 0.15 \). Similar to the motor symptoms, pain showed a strong, negative statistically significant correlation with MTR values, but no correlation with cross-sectional area.

**Table 7.** Spearman’s rank correlations

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Spearman’s Rank Correlation Coefficient (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTR Values</td>
<td>Cross-Sectional Area</td>
<td>-0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean MTR Values</td>
<td>Sensory Symptoms</td>
<td>-0.71</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean Cross-Sectional Area</td>
<td>Sensory Symptoms</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean MTR Values</td>
<td>Motor Symptoms</td>
<td>-0.87</td>
<td>0.03*</td>
</tr>
<tr>
<td>Mean Cross-Sectional Area</td>
<td>Motor Symptoms</td>
<td>-0.58</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean MTR Values</td>
<td>Pain Symptoms</td>
<td>-0.87</td>
<td>0.03*</td>
</tr>
<tr>
<td>Mean Cross-Sectional Area</td>
<td>Pain Symptoms</td>
<td>-0.58</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Discussion**

**Magnetization Transfer Imaging**

Based on the descriptive statistical analysis, the mean MTR value of the cervical cord for the control group was larger than the mean MTR value for the patients, which is consistent with previous findings \(^{48,49,53}\). Although MTI analysis is not a specific indicator of myelin content, MTI does reflect changes in myelin, such as demyelination \(^{44}\). This suggests that the patient group has demyelination in the cervical spinal cord, because a reduced MTR values are indicative of changes in myelin \(^{19}\). These findings symbolize the general reproducibility of MTI analysis. As suggested
in previous literature, MRI provides some insight into changes in water content and thus inflammation, when in fact, MTI can be used to evaluate macromolecules associated with water, allowing for the pathological evaluation of demyelination. In a sense, MTI could be considered a biomarker for damaged myelin.

Examining MTR values at each vertebral level for the TM patient and MS patient compared to controls shed light on both the sensitivity and shortcomings of MTI analysis. In the TM patient, MTR values were significantly reduced in value compared to the controls group at six different levels. Of these six different levels, 50% corresponded to a region within the TM patient’s identified level of injury. On the other hand, in the MS patient, MTR values were significantly decreased in value compared to the control group at three different levels. Of these three different levels, MTI analysis was able to accurately identify 33% of decreased MTR values at the level of injury. These results suggest that perhaps MTI analysis is a good tool for evaluating the entire cervical spinal cord, but is less sensitive at the exact level of injury. This reflects a multiple comparison problem, in which the significant differences observed could be due to chance, meaning that they are false positive. Additionally, these results could be interpreted to mean that the TM and MS patient have decreased MTR values along the entire spinal cord that are not lesion specific.

**Cross-Sectional Area**

The mean cross-sectional area of the cervical spinal cord for the control group, was larger than the mean cross-sectional area of the patient group. These results are also consistent with previous findings, as one study deduced that there were lower spinal cord area in MS patients compared to controls.
Analysis of the TM patient’s spinal cord revealed no significant differences in cross-sectional area measures compared to the control group. This suggests that the cross-sectional area measurement lacks specificity at the location of the injury in the TM patient. In contrast, in the MS patient, the cross-sectional area of the cervical spinal cord was found to have significant differences in cross-sectional area measures at levels C4-C5 and mid C5. The level of injury of the MS patient was C4, so these findings suggest that reduced cross-sectional area occurs near the level of injury. Moreover, decreased values of cross-sectional area at the level of injury was more sensitive in the MS patient than in the TM patient. However, similar to the MTR value analysis, the cross-sectional area analysis also may be affected by a multiple comparison problem, resulting in significant difference that may have only been due to chance.

Correlation

Spearman’s rank correlation analyses revealed more sensitivity to statistically significant relationships between mean MTR values and clinical status than mean cross-sectional area and clinical status. A previous study measured that $T_1$ and $T_2$ spinal cord areas showed a weak to moderate correlation with three markers of clinical status in MS patients. In this analysis, there was a strong, negative correlation between mean MTR values and motor symptoms. Similarly, there was a strong, negative correlation between mean MTR values and pain symptoms. There was a weak, negative correlation between mean MTR values and sensory symptoms. A negative correlation suggests that as MTR values decreased, symptoms increased. This illustrates that low MTR values, implicating demyelination, are associated with an increase in clinical presentation of symptoms. A previous study demonstrated that spinal cord MTR values weakly correlated with EDSS scores in patients with MS. Another study examined the measures of MTR values of the dorsal and ventrolateral spinal cord and its association with disability scores based on American
Spinal Injury Association (ASIA) impairment scale in patients with chronic cervical SCI. The researchers concluded that MTR values in the dorsal spinal cord predicted sensory disability, whereas MTR values in the ventrolateral spinal cord predicted motor disability. In contrast to the previous studies that found primarily weak correlations between MTR values and clinical status, the results from this study must be interpreted with caution. These results lack statistical power, which as a consequence lowers the chances of a statistically significant result uncovering the true effect. In this case, the effect size may be blown out of proportion because the values are based on two patients. Regardless, the idea that there is some type of relationship between MTR values and clinical status in a pediatric population, which is consistent with previous research done in an adult population.

**Other Variables**

There are several variables that may have played a role in the observed results. First of all, the difference in the patients’ disease as well as the time frame of the scan should be taken into consideration. The TM patient was scanned during the acute phase, or the period of time within a month of initial diagnosis, while the MS patient was scanned during the sub-acute phase, or the period of time within a year of the initial diagnosis, but after the first month. This difference in time and disease progression in the two patients may have accounted for the differences between the patients’ imaging data. For instance, the MS patient had enhanced specificity to the level of injury in the cross-sectional area analysis compared to the TM patient. It is possible that cross-sectional area analysis is more sensitive after the initial month of diagnosis and cannot be detected immediately following onset of disease. However, it could also be interpreted as a disease characteristic that differentiates MS from TM. In contrast, the acute TM patient had more sensitivity to MTR values being significantly different than controls at the level of injury compared
to the MS patient. This could mean that lesion identification through MTR analysis is superior immediately following diagnosis. Moreover, cross-sectional area measures appear to be more sensitive in the sub-acute MS patient, whereas the MTR values are more sensitive in the acute TM patient. Nevertheless, these differences may have only been due to chance, highlighting the need for a larger sample size to be examined.

Another variable that should be assessed in the examination of these two patients is disease characteristics. Both patients had low serum 25-Hydroxyvitamin D concentrations, 23.2 ng/mL in TM and 26 ng/mL in MS. Therefore, the MS and TM patient may have been treated for low vitamin D levels prior to the research scan, which may be a confounding variable. Likewise, both patients were treated with corticosteroids, prior to the research scans. Previous literature has shown an association between vitamin D insufficiency and TM. Additionally, vitamin D levels have been shown to be associated with subsequent relapse rate in MS children. The MS patient had a BMI of 26.1 kg/m², which is in the overweight category. Research has shown that MS patients have higher BMI percentiles compared to controls. These disease characteristics align with previous research studies of pediatric patients with TM and MS.

Gender was another variable that may have impacted the outcome of the results in this study. The MS patient and the TM patient were both males, while all three of the controls were female. Although gender differences were not specifically evaluated in this study, gender may have been a confounding variable because it is possible that the manifestation of disease is different between genders. Recently, TM was estimated to affect females more than males, (1.9:1). Therefore, the absence of diversity in gender between the patient group and control group should be taken into account when assessing the results.
Limitations

Many limitations were encountered while performing this study. The most prominent limitation was the small sample size. TM is a rare disease. Approximately, 1 to 8 cases per million people in the United States report incidence of TM \(^{22}\). Therefore, in the three-month time frame designated for thesis data collection, only one patient with TM was recruited and scanned. Even though MS has a relatively higher prevalence compared to TM, a lower rate is seen in pediatric patients when compared to adults. Therefore, a patient with MS was added to the analysis. Consequentially, the small sample size used in this study resulted in lower statistical power, and perhaps distorted effects in the analyses. This may have caused either type I or type II errors to occur.

Using quantitative imaging techniques to assess the spinal cord is not yet a routine practice in clinical application due to the challenge of the analysis. The spinal cord is relatively small in size compared to the brain \(^{44}\). One reason that there is such limited information in regards to MTI analysis pertains to the difficulty in obtaining good quality images for analysis and then the challenge of actually performing the necessary techniques. The analysis is tedious and time consuming, which may be another factor as to why it has not been adapted for clinical use. However, the results generally remain consistent with previous findings in adult patients; therefore, if good quality images are obtained, this technique is feasible and appears promising for clinical use.

Another challenge that was encountered was the lack of availability of EDSS scores and ASIA impairment scores for both the TM and MS patient. This was due to the limited amount of information reported by physicians in patient’s medical records and the inconsistency between clinical documents. Additional challenges include one patient was removed from the overall study.
because he could not tolerate the scanner, and other patient’s data were discarded because of poor image resolution caused by dental braces.

**Conclusions**

In the future to address these limitations, studies should include more patients and controls to assess the differences and relationships between cross-sectional area, MTR values, and clinical status in TM and MS patients. This solution addresses the main limitation encountered during the study, a small sample size. Further, a widely used assessment scale for disability, such as, ASIA or EDSS, should be administered to more accurately classify clinical status based on a well-established classification scale. A larger sample size will increase the statistical power of the analysis to detect differences between patients and controls as well between patients with different demyelinating diseases. Additionally, future researcher should examine how MTR values, cross-sectional area, and clinical status change as the disease progresses.

The present work contributes to the field because it is the first study to compare the differences and relationships between MTR values, cross-sectional area, and clinical status in pediatric patients with TM and MS. The findings in this study reveal that MTI analysis is a feasible technique in detecting differences in the entire cervical spinal cord in patients compared to controls. However, MTI analysis used to identify the exact level of injury is only partially accurate, which may have been due to chance. Another important finding was that cross-sectional area measures were more sensitive to differences in the MS patient compared to controls, while MTR values were more sensitive to differences in the TM patient compared to controls. These results may suggest that progression of the disease, as either acute, sub-acute, or chronic, may contribute to the observed differences between the patients. It is also possible that the specific disease MS or TM caused these results. Further research should be done to determine the underlying causes of
these observed differences between the patients. Lastly, this study demonstrates that there is a relationship between MTR values and clinical status in pediatric patients. This is important because this knowledge could be used in the future by medical professionals to determine better treatment plans for patients.

It is known to be difficult to accurately diagnose TM and MS patients based on clinical status. Patients present with a wide array of both motor and sensory deficits that can easily be confused with other demyelinating diseases. This manifests the need to find a better tool to properly diagnose TM and MS patients. Fulfilling this need will ensure that patients receive the appropriate treatments to help improve the outcomes of the disease.
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References