

Utilizing a novel T1rho method to detect spinal degeneration via magnetic resonance imaging

David Wang¹, Ping Wang²

¹Gilbert Classical Academy, Gilbert, Arizona

²Barrow Neurological Institute, Phoenix, Arizona

SUMMARY

Spinal degeneration has been linked to critical conditions such as osteoarthritis in adults aged 40+; while this condition is considered to be irreversible, we took interest in magnetic resonance imaging (MRI) for early detection of the condition. Ultimately, our purpose was to determine the effectiveness of a relatively novel T1rho method in the early detection of spinal degeneration, and we hypothesized that the early to mild progression of spinal degeneration would affect T1rho values following an MRI scan. This research utilized increasing trypsin injection dosage (0.1–0.2 mL/kg), an enzyme known to artificially simulate spinal degeneration, in the discs of a swine spinal specimen (a standard spinal cord injury model) to observe the stages of spinal degeneration at a faster pace. As it is known that increased trypsin injections would directly advance spinal degeneration, we corresponded 0.1 mL/kg trypsin-treated discs to early degeneration and 0.2 mL/kg to mild degeneration. We then scanned the treated swine spine using MRI and analyzed its quantitative T1rho (spin-lattice relaxation time) values at advancing stages of spinal degeneration. We found that T1rho values from the MRI did increase from 0.1 mL/kg of trypsin (early degeneration) to 0.2 mL/kg (mild degeneration). We were able to identify a direct correlation between T1rho values and progressing stages of spinal degeneration. Because other methods such as T1 mapping, T2 mapping, and diffusion imaging have faced limitations in diagnosing spinal degeneration, this T1rho method could prove valuable to future research and diagnosis of spinal degeneration.

INTRODUCTION

Spinal degeneration has become increasingly prevalent, with advanced forms often leading to irreversible conditions such as severe scoliosis (1). This debilitating condition is characterized by spinal disc deterioration, often naturally due to age as biological compounds such as proteoglycan gradually decrease in the spine (2). Particularly with adults over the age of 40, spinal degeneration can be present in the form of back pain or occur with no clear symptomology (3). Critically, this troubling circumstance that spinal degeneration can be symptomless highlights the need for developing an effective diagnosis technique for spinal degeneration prior to advanced stages.

Studies have incorporated various magnetic resonance

imaging (MRI) parameters and factors in attempting to detect spinal degeneration. Delayed gadolinium-enhanced MRI of cartilage and sodium MRI are methods that have been proposed as quantitative measures, but they faced severe limitations in utilizing effective contrast agents (4). Notably, research has been made in observing degenerative bovine spinal discs via magnetic resonance spectroscopy (SVS). These studies indicated that utilizing SVS parameters in MRI imaging was a somewhat effective method for detecting degeneration of intervertebral discs but encountered many limitations that hindered applicability to humans; minimal disparities between lipid peak lactate in relation to SVS and the bovine spine's structural differences to those of humans among other issues contributed to the conclusion that SVS is ultimately an unreliable method for quantifying spinal degeneration (5).

However, spinal degeneration is a physical condition that can lead to serious afflictions if untreated, such as osteoarthritis and spinal stenosis (6). While spinal degeneration itself has been established as a critical issue, radiological imaging has proven challenging; specifically, the term “degeneration” can be subjective among researchers and comprehension of its pathophysiology in the human spine is crucial to diagnosis (7). Therefore, we aimed to utilize objective and quantitative measures of investigating spinal degeneration through the T1rho values, in comparison to other methods of diagnosis that have faced major limitations.

T1rho is a form of relaxation time, a metric measuring hydrogen nuclei relapse rate of tissue after MRI pulses quantified, via MRI (8). Unlike T1 and T2 weighted contrasts or relaxation times of MRI, T1rho acts as a tissue parameter that has proven useful for observing chemical shifts. Specifically, T1rho was a value of interest to us because of its viability in detecting proteoglycan (PG) reduction, a macromolecule that can be present and subject to changes in the spine, specifically the intervertebral disc where degeneration occurs (9). Consisting of chondroitin sulfate and keratin sulfate in glycosaminoglycan chains, PGs play a crucial role in various tissue properties and stability (10).

We utilized T1rho, a parameter suitable for MRI analysis, to quantify spinal degeneration in swine spinal cords and determined its effectiveness in quantitatively representing chemical changes in proteoglycan content as spinal degeneration progressed. T1rho values were measured at various spin-lock frequencies (FSLs), which are MRI pulse sequences that can produce different saturated contrast levels for observation (11). By incorporating swine spinal samples, a close alternative to humans, we deduced that the results could be applicable for patient diagnosis. In this study, we hypothesized that quantitative T1rho values could be utilized from MRI scans for early detection of spinal

degeneration. Specifically, we hypothesized that T1rho values would correlate with shifts in proteoglycan, which generally decreases as spinal degeneration advances (12).

The natural enzyme trypsin was incorporated into this study, as research has highlighted its viability in observing spinal degeneration as an artificial catalyst for the condition; specifically, this enzyme can degrade the surface proteins of cells such as those in the spine, leading to cell death as an indicated factor in the progression of spinal degeneration (13). To exemplify, studies have observed trypsin injections in bovine spinal samples, also a substitution for humans. However, while the bovine samples were impacted by trypsin in certain parameters such as the neutral zone (a measure of spinal relaxation), the practicality of the experiment was unclear as the human spine differs greatly from that of cattle (14, 15). Thus, the results involving a swine spinal specimen in this study would prove more applicable, as their anatomical structures are more closely related to that of humans; notably, pig spine incorporates a similar cervical vertebra to that of humans, proving it a viable substitution (16). During this experiment, the individual discs in a pig spinal specimen were tested by injecting trypsin at varying levels.

RESULTS

Ultimately, this study aimed to evaluate T1rho imaging in response to spinal degeneration, which was stimulated by trypsin as an artificial catalyst. To test this, we employed a Philips 3-Tesla MRI scanner that operates through a strong magnet and radio waves, while connecting with a scanner console to generate images (17). This apparatus has already shown success in detecting numerous anomalies, notably brain tumors, multiple sclerosis, and dementia (18). We also utilized external programs like the ImageJ program to determine T1rho values.

Three spinal discs were treated with 0.1 mL/kg of trypsin to mimic early degeneration, three discs were treated with 0.2 mL/kg of trypsin to mimic mild degeneration, and three discs were treated with 0.2 mL/kg of water to act as the healthy control group. T1rho values were taken at various FSLs from

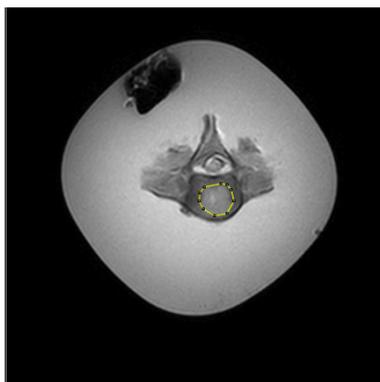


Figure 1: Identification and isolation of the spinal nucleus pulposus via ImageJ. Image showing a trypsin-treated spinal disc from top view (axial slice). The yellow region of interest (ROI) was created using ImageJ, and the area inside consists of the nucleus pulposus of the spinal disc, where the trypsin was injected and degeneration took place (48 hrs post-injection). T1rho values were extracted from the ROI on the T1rho map using ImageJ. It should be noted that the approximate diameter of each disc is 2 inches, and the overall approximate length of the spinal specimen is 8.5 inches.

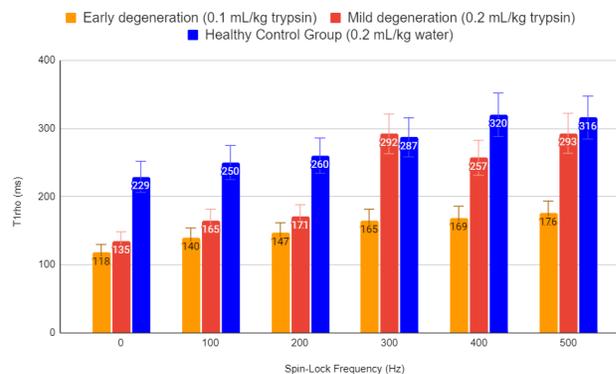


Figure 2: Bar graph showing the changes in T1rho at different spin-lock frequencies (FSLs) in comparing early degeneration, mild degeneration, and the control group (n = 3). T1rho maps were generated at various FSLs from 0 to 500 Hz using MATLAB, and the calculated T1rho values (ms) were expressed as an average by inputting a region of interest with ImageJ. All calculated T1rho values represent an average of three discs for each trypsin treatment of 0.1 mL/kg and 0.2 mL/kg, as well as the control group treatment of 0.2 mL/kg water. The error bars represent standard deviation of the data.

0 to 500 Hz for the purpose of viewing and analyzing them at different MRI contrast levels, with higher FSLs generally corresponding to higher MRI contrast as represented by ImageJ (Figure 1). With FSLs commonly utilized in MRI to evaluate T1rho values in similar studies, we also used FSLs as a standard metric to generate and observe T1rho values (19).

We found that at an FSL of 0 Hz, the average T1rho values increased in correlation with degeneration from the early (0.1 mL/kg trypsin) to mild (0.2 mL/kg trypsin) stages, from 118 to 135 ms (Figure 2). Similarly, when compared to higher contrasts at an FSL of 500 Hz, the average T1rho values also notably increased from 176 ms at early degeneration (0.1 mL/kg trypsin) to 293 ms at mild degeneration (0.2 mL/kg trypsin). We were able to conclude that, when paired with MRI in detecting spinal degeneration, T1rho values increase with degeneration in intervertebral discs in our swine spine model. However, the average results from the control group (0.2 mL/kg water) were quite unexpected. We discovered that the control group's average T1rho values across all FSLs from 0 to 500 Hz were generally higher than trypsin-treated, degenerative spinal discs at both the early and mild stages. For example, at 400 Hz, the average T1rho value for the control group was 320 ms compared to 169 ms at early degeneration and 257 ms at mild degeneration.

DISCUSSION

Ultimately, we found that T1rho values increased between early degeneration and mild degeneration stages of our swine spinal model, from FSLs 0 to 500 Hz. The control group, in general, did unexpectedly produce higher T1rho values than the experimental groups. Having hypothesized that T1rho, when paired with MRI as a quantitative value, is affected by spinal degeneration, we found our results to generally support this idea. However, because this experiment could not be repeated further for statistical analysis due to limited spinal samples, all data can be considered preliminary. While

certain limitations were present, we compared this study to other methods and determined to the best of our knowledge that T1rho is highly effective compared to T1 mapping, T2 mapping, and diffusion imaging for detection of spinal degeneration. To exemplify, while T1 mapping has been able to represent shifts in water levels in spinal discs, this method faces a crucial restraint as T1 values demonstrate little change in the early stages of a disease, even when combined with the cell signal intensity of MRI (20, 21). Furthermore, T2 mapping is a similar method to T1, though it encounters a major limitation as studies have demonstrated little connection between this transverse relaxation rate ($1/T_2$) and proteoglycan changes during degeneration (22, 23). Finally, diffusion imaging is a method that is fairly effective in detecting spinal degeneration, with its apparent diffusion coefficient values (measure water diffusion in tissue) shown to decrease in correlation with intervertebral disc deterioration; however, it is also limited in detection capabilities as diffusion imaging cannot effectively discern between afferent and efferent structures of the nervous system, which direct neural impulses to the brain and muscles, respectively, and may be affected in the spine during degeneration (24, 25, 26). Thus, as these alternate imaging methods are severely limited in evaluating degenerative changes, T1rho as a quantitative method may hold promise in comparison to other systems.

Certainly, we did discover limitations to using the T1rho method. The water used for the control group injections was tap water from the lab, so it is possible that external factors such as irregular pH would have influenced T1rho results of the water-treated spinal discs, by denaturing enzymes present in the spinal discs (27, 28). Extreme pH can modify the protein surface charges of tissue, potentially impacting T1rho readings of the discs (29). Critically, an error was likely present in the control group as it demonstrated an immense shift in T1rho, which was quite different compared to similar research that, albeit incorporated phosphate-buffered saline injections instead of water for the healthy disc control group, revealed T1rho values that were generally below 200 ms, even during the pre-experimental phase (30). In contrast, T1rho values for the water-injected disc control group in this experiment remained above 200 ms when measured at various FSLs.

If this study were to be expanded upon in the future, we would take multiple steps to strengthen reliability in detecting spinal degeneration via T1rho. One potential change is that the control group of spinal discs would instead not be injected with any compounds such as water, to ensure that external factors do not result in chemical shifts. Furthermore, certain solutions such as phosphate-buffered saline have proven more relevant and effective than water as a control injection, by preserving the hydration of tissue prior to and during testing efficiently (31). We also hope to utilize a larger sampling quantity, including an increased amount of trypsin at 0.3 mL/kg among a group of spinal discs, in order to observe degeneration at an advanced stage in relation to T1rho detection. Furthermore, although proteoglycan content theoretically decreases during spinal degeneration as highlighted in this study, we would hope to verify this via means of precisely measuring proteoglycan such as safranin-o staining. Finally, as degeneration was artificially stimulated in this study via trypsin injections in swine spinal discs, incorporating human patients with disc degeneration

would always prove more accurate and relevant.

In conclusion, we found that utilizing T1rho as a quantitative measurement demonstrates promise compared to other proposed methods of detecting spinal degeneration, such as T1 mapping. Specifically, although further research involving a greater degree of trials and means of simulating spinal degeneration beyond trypsin would be required to solidify the results, a correlation was discovered in which T1rho values increased as spinal degeneration advanced from early to mild stages. Therefore, this connection illustrates that T1rho values may increase from diminished spinal proteoglycan content due to degeneration, highlighting a potential inverse relationship. From this study, we hope that further research involving T1rho's viability for detecting spinal degeneration in human patients could be performed to attain a more quantitative and objective means of diagnosing this condition. If such a method were solidified, the early detection of degeneration in spinal discs could prompt preliminary treatment to prevent the condition from progressing into scoliosis and other spinal diseases of an incurable nature.

MATERIALS AND METHODS

Preparing the swine spinal specimen for MRI

To initiate the study, we began by obtaining a swine spinal sample from the Barrow Neurological Institute and stored in phosphate-buffered saline (PBS) to reduce dehydration. The swine specimen used was a male Hanford Miniature pig approximately five months old, used for lab research purposes, and the lumbar region of its spine was used. Nine disc sections were then located on the swine spinal specimen. Using sterile injection needles (27G), three consecutive discs from the lumbar spine were each injected with 0.1 mL/kg of trypsin (25 mg/mL, Sigma Aldrich) in the nucleus pulposus to represent early degeneration, 0.2 mL/kg of trypsin to represent mild degeneration, or 0.2 mL/kg of water for the control group. Once the swine spinal discs were treated with trypsin, the specimen was placed vertically in the PBS container at 4°C for 2 days, allowing for the trypsin to take effect and artificially simulate spinal degeneration.

Adjusting MRI settings prior to experimentation

The MRI machine used in this study was a Philips 3-Tesla scanner. We placed the container with the treated pig spinal specimen inside the apparatus and initiated the MRI scan, which lasted approximately 4.5 minutes per individual spinal disc (**Figure 3**). Regarding imaging settings on our scanner console, the field of view (visual range in which image is shown) was 245 x 245 mm. The pixel size (to influence image resolution) was 0.5 x 0.5 mm. The slice thickness (width of image) was 4 mm. The Repetition Time/Time to Echo (time between MRI pulses/time between RF pulse and echo signal) was 3000 ms/10 ms. The Turbo Spin Echo Turbo Factor (number of echoes received following MRI excitations) was 15. The Number of Excitations (an MRI parameter) was 1. The spin-lock times (average times of MRI relaxation) were 1 ms, 11 ms, 21 ms, 31 ms, and 41 ms. FSLs were taken at 0 Hz, 100 Hz, 200 Hz, 300 Hz, 400 Hz, and 500 Hz.

Data Collection of T1rho values

MRI images of each spinal disc were transferred to the MATLAB programming language, allowing MRI-generated

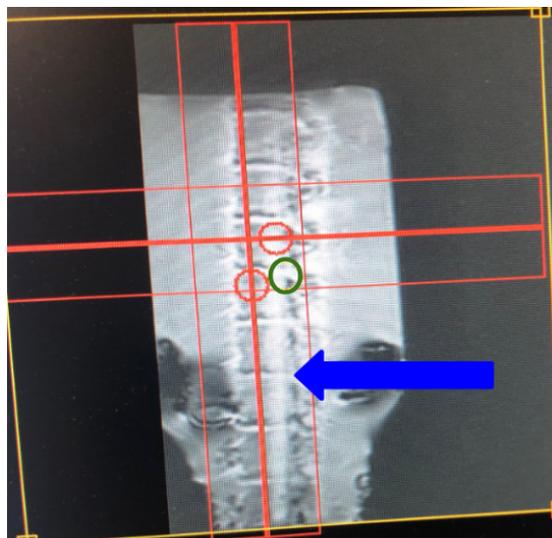


Figure 3: General MRI scan of swine spinal specimen post-treatment. MRI image showing a sagittal slice of the swine spinal discs utilized for the study taken at pixel size: 0.5 x 0.5 mm. The band like structures on the spine are the spinal discs (indicated by the blue arrow), in which degeneration occurred following trypsin injections at 0.1 and 0.2 mL/kg. The red and green circles indicate the centers for position, as the MRI parameters were adjusted.

images to be optimized to T1rho maps. T1rho values were simultaneously calculated for these T1rho maps. Contrary to misconceptions, T1rho is not directly expressed by MRI imaging itself. This value was calculated in our study using a combination of external factors including the MATLAB programming language and ImageJ program. In contrast to other programming languages such as Python and Java, we utilized MATLAB in our study for its ability in studying image processes, essentially optimizing the imaging from MRI for further research (32). Then, the ImageJ program is utilized with MATLAB images and allows for the isolation of a region of interest on a particular image, by digitally creating a perimeter around that area before expressing applicable values that MATLAB has calculated, such as T1rho. ImageJ was used to identify and highlight the nucleus pulposus on each spinal disc's T1rho map, and the corresponding T1rho values in those areas were then generated onto a spreadsheet. T1rho values of each spinal disc were calculated and averaged in accordance with their treatment type.

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