Quantitative Magnetic Resonance and Histological Analysis of Primary Motor Neuronal Loss in Amyotrophic Lateral Sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder primarily involving the motor neurons in the cerebral cortex, brainstem and spinal cord (1). Most cases of ALS occur sporadically and are of unknown etiology such that the progression of the cellular events in the nervous system from initial insult to final cell death is unknown. Loss of neuronal function within the primary motor cortex (PMC) is common in late stage ALS with neurons in the upper motor region having already been profoundly affected when diagnosis is made solely on clinical manifestation of the disease (2). An understanding of the etiology and sequence of neuronal loss seen in ALS would aid in monitoring the advancement of the disease. Here we present data on the evaluation and quantification of MRI T_1 and T_2 image and parameter mapping differentials found in the primary motor cortex of ALS cadaver brains.

Methods: Four cadaver brains of ALS patients were excised during morning autopsy, bisected sagittally and one half of each fresh brain was then embedded in Alginate dental impression polymer. This material maintains hydration, rigidity and immobility of tissue sample during imaging. A T_1 weighted 3D image set with a 20 x 20 x 6.0 cm FOV, 256 x 256 x 60 matrix, 25.33ms TR, 4.8ms TE, 8 NEX was obtained in 140 minutes. Images for T_1 and T_2 measurements were also acquired with similar geometric parameters using a spin echo saturation-recovery sequence. T_1 and T_2 parameter maps were created using using Cincinnati Children's Hospital Image Processing Software (CCHIPS). After completion of scanning protocols, the brain tissue was fixed in 10% Low Odor Formalin for one week. Following fixation, gray matter from the primary motor cortex, frontal lobe and occipital lobe were excised, cryogenically protected with graded sucrose solution and sectioned at 20 µm thick with a Leica cryostat. Sections were stained for neuronal density with a Nissl cresyl violet stain which is commonly used to stain Nissl bodies in the cytoplasm of neurons for identifying the basic structure in brain.

Results: As seen in Fig. 1, superior T_1 contrast between gray and white matter within the primary motor cortex is markedly lower than those in other brain areas. All four cadaver brains studied exhibited the same lack of gray and white matter contrast in PMC as compared to all other regions of the brain. This is illustrated in Figure 1 by the arrow pointing to the top of the PMC. T_1 and T_2 parameter mapping and region of interest analysis show significant differences in relaxation values. Average T_1 relaxation values for gray matter within the primary motor cortex were 1220ms, 1405ms for the frontal lobe, 1470ms for the occipital lobe and for white matter within the internal capsule 1007ms. Average T_2 relaxation values for the primary motor cortex were 89 ms, frontal lobe 103ms, occipital lobe 100 ms and white matter 134ms as seen in Figure 1. The Nissl stain (Figure 2) shows a lack of contrast differential in the motor cortex with a loss of neuronal density in the primary motor cortex as compared to the frontal and occipital gray matter. The lack of contrast differential seen between white and gray matter in the PMC does not seem to be due to cortical thinning in the region, as the thickness of the gray matter in the PMC is comparable to the other gray matter regions.

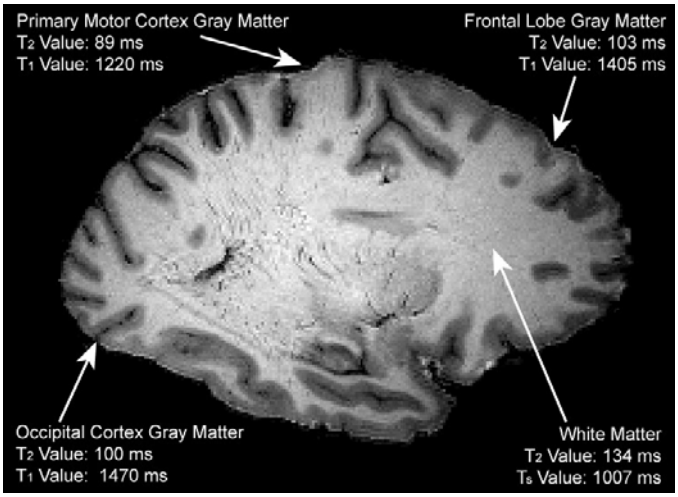

Figure 1: T_1 weighted image of an ALS cadaver brain with T_1 and T_2 relaxation values. Regions of interest for the gray matter of the primary motor cortex (top left arrow), frontal lobe (top right), occipital lobe (bottom left) and frontal white matter (bottom right) were selected and relaxation averages obtained. The T_2 value for PMC was 89ms, frontal lobe 103 ms, occipital lobe 100 ms and white matter 134 ms. The results indicated a difference in gray matter relaxation within the PMC compared to other gray matter regions.

Discussion: MRI images of freshly excised cadaver brains show a lack of gray/white matter boundary contrast within the primary motor cortex, when compared to occipital and frontal lobe gray matter regions. Magnetic resonance T_1 and T_2 parameter mapping of the cadaver tissue allows for accurate quantification of contrast differentials in the motor cortex of ALS tissue. This contrast differential is significantly different from other brain regions of interest and when evaluated with the Nissl staining protocol it was found that neuronal density within the PMC is likely responsible for the resulting lack of MR contrast between white and gray matter. Cortical thickness of the PMC does not seem to be solely responsible for the contrast differential. A marker of ALS disease severity in the upper motor neurons would aid in the early diagnosis of the disease. These results provide insight into the potential benefits of using T_1 and T_2 parameter mapping to accurately quantify relaxation values in ALS tissue. Further study will employ the usage of Glial Fibrillary Acidic Protein staining to verify neuronal loss and glial / astrocyte proliferation in the histological tissue analysis.

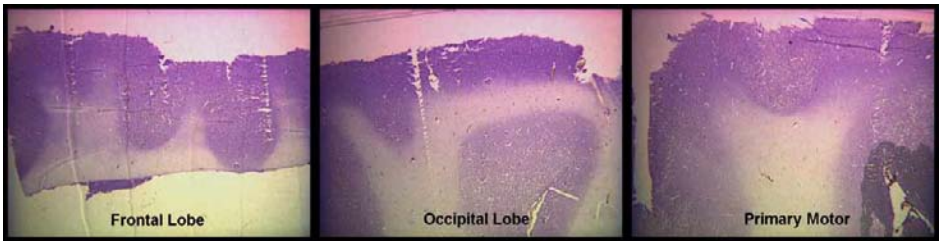

Figure 2: Nissl stained 20 µm thick tissue samples of the frontal lobe (left), occipital lobe (middle) and primary motor cortex gray matter (right). All three images were taken at the same magnification (10x) and image intensity for comparative purposes. The frontal and occipital lobes exhibit more neuronal density than the primary motor cortex. The lack of contrast seen with the MR images corresponds to the lack of neuronal density but not to cortical thickness. The cortical thickness of the three regions seems comparable to one another.

References:

1 – Hong *et al*., Jorurnal of Neurological Sciences 2004; 227: 73 – 78

2 – Abe *et al*,. NMR in Biomedicine 2004; 17: 411 – 416.