The Relationship between Vitamin D Serum Level and Midsagittal Corpus Callosum Area in Patients with Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a disease with a pathologic pattern marked by focal white matter demyelinative lesions and neural degeneration in the brain and spinal cord. Corpus callosum (CC) thinning and brain atrophy are the visible endpoints of irreversible tissue loss in MS, arising from multiple pathologic mechanisms. Research has uncovered a relationship between low circulating levels of vitamin D as it relates to MS susceptibility and severity, suggesting that vitamin D has neuroprotective properties. This study assesses the relationship between 25-hydroxyvitamin D levels and midsagittal CC area in a sample of 33 adults diagnosed with relapsing-remitting MS. We parcellated the CC into five distinct segments (genu, rostral body, midbody, isthmus, and splenium) to determine a more specific locus of white matter change as a function of vitamin D level. Co-varying for age, years diagnosed, and education level, results indicate splenium CC area is positively associated with 25-hydroxyvitamin D level. This finding confirms the possible importance of vitamin D in the pathogenesis of MS and the therapeutic potential of vitamin D for patients.

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1   Introduction

Multiple sclerosis (MS), a disease affecting several million people worldwide with no available cure, manifests in both physical and cognitive deficits, including fatigue, loss of sensation, limb weakness, slowed mental processing speed, and impaired long term memory. Multiple cell types are involved in what is considered a complex autoimmune and neurodegenerative disease marked by chronic inflammation of the central nervous system. A decrease in axonal insulation from demyelination causes at least transient compromise of neural communication, with the traditional view being that tissue destruction results from an inflammatory immune response directed against myelin. Yet, recent findings suggest that early lesions in MS may be non-inflammatory in character. In the medulla, for example, lesions bordering normal-appearing white matter (NAWM) show no infiltrating inflammatory components yet demonstrate apoptotic oligodendrocytes prior to removal by macrophages [1-3]. Thus, it appears that neuronal lesions in MS may be both independent of inflammation or secondary to inflammation. Enduring demyelination begins a process of Wallerian degeneration in which the affected axon atrophies distal to the point of insult [4]. With structural magnetic resonance imaging (MRI), this degeneration is often notable in the corpus callosum (CC) in part due to the large number of myelinated fibers which it contains [5].

Research in the last decade indicates that vitamin D has immunomodulating properties, and there is replicated evidence that vitamin D metabolism plays some role in individual susceptibility to MS. Vitamin D has been shown to affect brain development and function, cell proliferation and apoptosis, immune cell differentiation , and a modulation of immune responses [6, 7]. Several key components of the immune system express a subtype of the steroid-receptor super family called the vitamin D receptor (VDR). Such components include monocytes, dendritic cells, B-cells, CD4$^+$ T-cells, cytokines, and macrophages [8]. The transcription, proliferation, and differentiation of each of these immune cells changes depending on circulating vitamin D levels [9]. Although the mechanisms are not fully elucidated, research suggests that vitamin D helps to establish CD4$^+$ T-cell and cytokine equilibrium [for review, see 10], prompting MS clinicians to support vitamin D supplementation as a safe and beneficial therapeutic approach.

Preliminary research from the epidemiological and experimental literature supports this idea. For example, Correale, Ysrraelit, and Gaitán [11] found that vitamin D levels were significantly lower in relapsing-remitting MS patients compared to control subjects. Moreover, patients during relapse had significantly lower levels of vitamin D metabolites than during remission. In an intervention study, Burton and colleagues [12, 13] found that patients receiving high doses of vitamin D experienced significantly fewer relapses than those in a non-treatment control group. Corroborating this, a recent prospective study found that, with each doubling of 25(OH)D concentration, relapse rate decreased by 27% in patients [14]. Animal studies, which have far fewer confounds than human studies, have produced converging results. Cantorna, Hayes and DeLuca [15] found that large 1,25(OH)2D administration prior to experimental autoimmune encephalomyelitis-induction (EAE, the animal model of MS) prevented animals from developing the disease. EAE mice who were fed diets high in vitamin D presented with fewer physical symptoms of the disease relative to non-supplemented EAE mice, and,

when vitamin D was removed from the diet, these mice quickly started to develop new physical MS symptoms. Taken together, these results suggest that vitamin D works directly to prevent and alleviate MS symptoms.

There remain many unanswered questions about vitamin D and MS, including the specific mechanisms by which vitamin D influences the course of MS. Further investigations have relied on short-term MRI outcomes that are not necessarily indicative of long-term disease status, cognition, or neural anatomy. The present study addresses this gap in the literature by investigating the relationship between white matter brain changes in the corpus callosum (CC) and vitamin D serum levels in individuals with relapsing-remitting MS. Because little information exists on the precise locality of CC degeneration in MS, we parcellated the CC into five distinct midsagittal segments in order to determine a more specific locus of white matter change as a function of vitamin D level. In light of the literature that suggests a neuroprotective role of vitamin D, we expected a positive relationship between CC subregion area and vitamin D blood serum levels.

2   Methods

The sample included a total of thirty-three (28 women), White/non-Hispanic, right-handed adults in the northern New England region (44 - 45°N) of the United States whose diagnosis of relapsing-remitting MS was confirmed as meeting McDonald diagnostic criteria [16] by a board certified neurologist experienced with the diagnosis and management of MS (M.K.R.). Age ranged from 26 to 69 yrs ($M = 47.9$, $SD = 11.7$), and educational achievement ranged from 12 to 19 yrs ($M = 14.1$, $SD = 2.2$), indicating partial college education on average. Duration of illness since diagnosis ranged from 2 to 32 yrs ($M = 9.3$, $SD = 8.4$), and all had an Expanded Disability Status Score (EDSS) less than 4.5. All participants were taking disease-modifying agents, and 81% of the sample was taking vitamin D supplements at the time of study. The protocol was consistent with ethical guidelines of the Declaration of Helsinki and was approved by the appropriate Institutional Review Boards. Written informed consent was obtained per participant.

Brain images were acquired using a GE Signa 1.5-T magnet with echo speed gradients using a standard RF head coil. Sagittal SE T_1, axial FSE T_2, axial and sagittal FLAIR (having same parameters), and axial diffusion-weighted images were obtained. Manual volumetry was conducted on the T_1 data, collected with the parameters: TR = 216.2 ms, TE = 2.6 ms, flip angle = 80 degrees, NEX = 2, and slice thickness = 5 mm, yielding varying contiguous slices dependent on head size with a 320 cm field of view and a 640 x 640 matrix.

Manual tracing was performed by two individuals (S.M. and M.H.C.), each blind to vitamin D serum status of the subject, using 3-D Slicer software, version 2.7 (www.slicer.org). Images were first aligned along the line of the anterior commissure and posterior commissure. Working within the sagittal plane, total CC area was delineated before parcellation in order to avoid partial area effects. The CC was subsequently divided into five subregions based on the Weis geometric partitioning scheme [17]. Using this method, the most anterior and posterior points of the CC were located, and the distance of the superior boundary between these points was calculated and divided by five. Along an artificially-created horizontal line between anterior and posterior points, six vertical lines were constructed to be perpendicular to the horizontal line. These equally-spaced tangent lines thereby dissected the CC into five subregions (Figure 1):

CC_1 (genu), CC_2 (rostral body), CC_3 (midbody), CC_4 (isthmus), and CC_5 (splenium). Total callosal area was calculated by adding together the values for the five subregions.
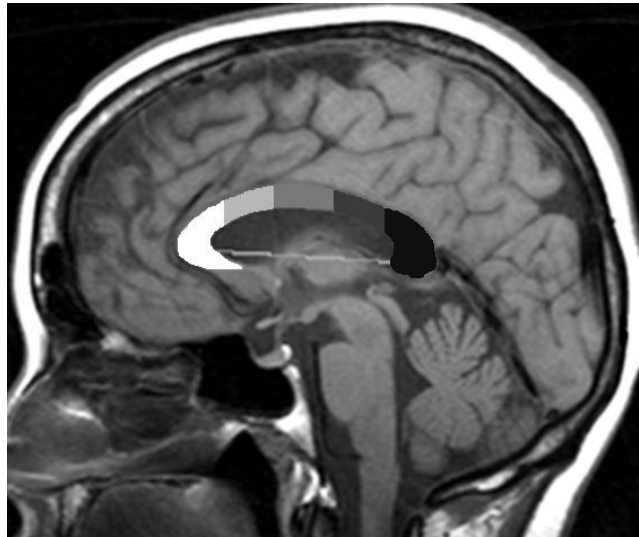

Figure 1: T_1-weighted MRI in sagittal view. Using the Weis method, the midsagittal corpus callosum was parcellated into five subregions. From left to right, lightest segment to darkest segment: CC_1 = genu, CC_2 = rostral body, CC_3 = midbody, CC_4 = isthmus, CC_5 = splenium.

Standard venipuncture was performed by a certified phlebotomist within close temporal proximity to the date of the participant's MRI scan. Vitamin D 25-Hydroxy levels were determined from fasting blood samples by the DiaSorin LIAISON® chemiluminescent immunoassay method at ARUP Laboratories (Salt Lake City, UT, USA), which has functional sensitivity of 4 ng/ml and a detection range of 4–150 ng/ml. Two levels of controls were run with a range of 10.3–20.3 ng/ml and 37.9–63.7 ng/ml.

3 Results

Inter-rater reliability for manual tracing of the total CC was .96. Total CC midsagittal area ranged from 217.8 to 642.2 mm^2 (M = 372.8, SD = 85.5). Using norms from Simon and colleagues [18] for healthy adult male and female midsagittal CC areas, we calculated z-scores per participant for each CC subregion. Z-scores for the total CC area ranged from -4.1 to 0.01 (M = -2.3, SD = 0.9), indicating that, on average for participants in this sample, total CC area was more than two standard deviations below that of a gender-matched healthy control. Likewise, CC subregion area z-scores fell between half and one standard deviation below what would be expected among healthy adults (Table 1). Vitamin D blood serum levels ranged from 14.7 to 114.1 nmol/L (M = 35.1, SD = 20.4). Based on National Institute of Health [19] criteria, 16 participants had deficient (< 30 nmol/L), 11 participants had insufficient (between 30 and 50 nmol/L), and 6 participants had adequate (> 50 nmol/L) levels of vitamin D. No participant had a serum value indicative of potential toxicity (> 125 nmol/L). These low-tending values are consistent with findings of vitamin D insufficiency in segments of the general population in far

northern climates [20, 21, 22].

A bivariate correlation analysis was conducted to evaluate the relationship between vitamin D blood serum level and the CC subregion area *z*-scores (Table 1). One-tailed test of significance was used, as directional effects were predicted *a priori*. CC subregion areas were strongly inter-correlated, and splenium area was positively associated with 25(OH) D level, $r(29) = .35$, $p = .03$. This latter effect held after a partial correlation that controlled for age, gender, education, and duration of illness, $r(23) = .41$, $p = .02$.

Table 1: Correlations with CC subregion area (mm^2) *z*-scores and vitamin D serum level (nmol/L)

	CC_1	CC_2	CC_3	CC_4	CC_5
Vitamin D	-.07(-.06)	.03(.05)	.13(.14)	-.17(-.19)	.35*(.41*)
CC_1		.45**(.37*)	.62**(.59**)	.56**(.53**)	.20(.13)
CC_2			.85**(.84**)	.76**(.71**)	.29(.10)
CC_3				.71**(.66**)	.41*(.33)
CC_4					-.10(-.24)
M	-.67	-.88	-.97	-.87	-.81
SD	.75	.48	.27	.50	.60

Note. Parenthetical values indicate the partial correlation, controlling for age, years of education, and duration of illness.
* $p < .05$
** $p < .01$

5   Conclusion

Consistent with the hypothesis that vitamin D is therapeutic and/or neuroprotective, we found a positive, significant correlation between serum vitamin D concentration and the midsagittal area of the splenium of the CC. This relationship held after controlling for demographic variables such as age, gender, years of education, and duration of illness. Vitamin D levels were not associated with midsagittal area of the genu, rostral body, midbody, or isthmus.

The splenium segment of the CC has fibers that connect one hemisphere to the other in posterior cortical regions of the occipital and parietal lobes. Using diffusion tensor tractography, Abe and colleagues [23] have shown that the interhemispheric connections from the caudal temporal lobe pass through the ventral part of the splenium. In this posterior fifth of the CC, superior temporal region fibers are arranged rostrally whereas the inferotemporal and preoccipital fibers are positioned more caudally. From a functional standpoint, the CC is the primary pathway for high-level cognitive integration across

hemispheres. Studies of interhemispheric communication in human and non-human animals have determined that the transmission of visual and sensory information between the hemispheres as well as likelihood of a well-developed memory trace system is optimal with commissural integrity in the splenium [24, 25, 26]. Functional MRI findings in MS patients have emphasized the potential for adaptive, compensatory cerebral reorganization within motor [27, 28] and cognitive networks [29] after onset of symptoms, and interhemispheric communication subserved by the CC may be essential for this plasticity. That the relationship between serum vitamin D level and CC area is localized to the splenium is not surprising in light of literature that suggests decreased structural connectivity in the splenium across multiple MS subtypes. For example, Giorgio et al. [30] found an inverse relationship between EDSS and fractional anisotropy (FA; direction of water diffusivity along axon tracts) in the splenium in adults with relapsing-remitting MS. Similarly, Bodini and colleagues [31] found that lower FA in the splenium predicts accelerated disease progression in patients with primary progressive MS. Among patients with secondary progressive MS who show T_1 enhancing lesions, splenium FA decreases over time [32]. Additional neuroimaging studies report functional changes in association with white matter structural pathology in the splenium. In a sample of patients with early-onset relapsing-remitting MS, Gadea et al. [33] found that significant, progressive loss of posterior CC areas, including the splenium, is related to increasing EDSS and unusual lateralization patterns on a Dichotic Listening task, an auditory test of interhemispheric transfer. In subjects with clinically-isolated syndrome suggestive of MS, verbal learning performance is inversely associated with presence of lesions in the splenium among other regions [34]. In a similar sample, Ranjeva et al. [35] found a low magnetization transfer ratio in the splenium to predict worse MS Functional Composite score and poor performance on the Paced Auditory Serial Addition Test, a measure of processing speed, attention, and working memory. Finally, Bethune et al. [36] reported that children with MS have lower splenium FA relative to age-matched peers and that FA value correlates with performance on neuropsychological measures of processing speed.

Our current finding of a positive relationship between vitamin D level and splenium area in relapsing-remitting MS subjects is the first such data and is important in that it confirms an association between vitamin D and the anatomy of fiber pathways in the human brain. However, the current finding must be considered in the context of the study's limitations. First, despite being a common clinical approach to evaluate serum vitamin D levels, questions have been raised as to the accuracy of immunoassay methodology such as DiaSorin [37]. While recent research suggests comparability in quantification of 25(OH)D from immunoassay methods and chromatographic methods such as liquid chromatography–mass spectrometry, immunoassay techniques are more prone to random error [38]. Second, given the modest sample size and imbalanced gender ratio of participants, we cannot evaluate whether there are sex differences in the relationship between vitamin D level and CC structure. Future studies with larger, more heterogeneous samples of patients with MS should replicate this finding and extend the scope of analysis to consider whether cognitive enhancement is also seen in conjunction with CC changes as a function of vitamin D. Diffusion tensor imaging will be a particularly useful methodology to determine if FA values in the CC and other white matter tracts co-vary with vitamin D levels.

Although the precise mechanisms by which vitamin D is neuroprotective are not well understood, initial evidence suggests a possible feedback mechanism between vitamin D and immune system functioning [8]. In addition to characterizing the anti-inflammatory

actions of vitamin D on CD4$^+$ T-cells, future research should assess if and how vitamin D regulates other cells with pathogenic contributions to MS, such as interleukin-17-producing T-helper 17 cells, B cells, and CD8$^+$ T cells [39]. Even in the absence of fully elaborated pathways, there is sufficient empirical support for continued excitement about the disease-modifying potential of vitamin D. Low-dose vitamin supplementation, however, may not be enough to raise circulating vitamin D to sufficient levels in individuals who live in northern climates, like those in our study. Less sunlight in these locations means less cutaneous synthesis of vitamin D. Compounding the problem, patients with motor impairment may spend less time outdoors, and patients with cognitive decline tend to have poor nutrition [40], thereby limiting introduction of vitamin D-fortified food into the diet. Given these considerations, practitioners should counsel patients with MS about lifestyle choices and effective year-round supplementation as behavioral measures to complement standard treatment regimens.

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