



Correlation between vitamin D and alterations in MRI among patients with multiple sclerosis

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Abstract

Introduction. Multiple sclerosis (MS) is a disease of unknown etiology. Diagnosis of MS is primarily based on detection of myelin damage by magnetic resonance imaging (MRI) and classification of demyelination according to the McDonald Criteria. Cholecalciferol (vitamin D₃) has been shown to affect the onset and progression of MS via its immunomodulating properties. The role of vitamin D in MS pathogenesis and treatment deserves further investigation, as there is sufficient evidence to suggest a correlation between vitamin D blood level and brain MRI lesion load.

State of knowledge. Elevated blood vitamin D concentration is linked with demyelination, as determined by T2-weighted and gadolinium-enhanced MRI. Blood vitamin D blood levels are affected by sun exposure, among other factors; however, there is no evident connection between abnormalities in myelination and seasonality. Vitamin D supplementation among MS patients has been associated with a lower probability of new lesions and loss of existing lesion volume, as observed seen in T1-weighted MRI scans ($p=0.03$). An increase in TGF-beta levels was noted among patients using vitamin D supplementation, which may suggest a mechanism by which cholecalciferol may improve MS prognosis. Patients with clinically isolated syndrome (CIS) exhibited an inverse correlation between vitamin D concentration and risk of new lesions as seen in T2-weighted MRI scans. Moreover, vitamin D intake among these patients lowered the risk of progression to clinically definite multiple sclerosis (CDMS).

Daily intake of vitamin D during fingolimod treatment correlated strongly with lower numbers of new lesions. High dose vitamin D supplementation during interferon beta-1a treatment was linked to lower mean percentage of lesions compared with volume pre-treatment seen by T2-weighted MRI.

Results. Recent findings advocate for the monitoring of vitamin D blood levels in MS patients. Vitamin D supplementation should be considered in both MS patients and patients with CIS, where other signs of disease may be delayed. Moreover, vitamin D supplementation appears to lower the likelihood of new demyelination changes apparent in MRI examinations.

Key words

Multiple sclerosis, Vitamin D, MRI, CIS

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease with an as yet undefined etiology. There are 2.5 million individuals with MS globally [1]. Factors which may affect the onset and progression of MS disease are still under investigation. Several genetic and environmental variables are associated with increased risk of MS, including bioclimatic factors, vitamin D levels, obesity, tobacco smoking and Epstein-Barr virus (EBV) infection. Nevertheless, the specific impact of each variable in isolation remains unclear [2, 3].

Magnetic resonance imaging (MRI) is a basic diagnostic tool commonly used for imaging of the brain to detect demyelinating lesions. Such lesions are seen at best resolution using T2-weighted and proton density (PD) sequences. MRI is used in monitoring of MS disease and, based on MRI images, MS is diagnosed according to the McDonald modified criteria [4, 5]. New MS neuroimaging criteria were proposed by Magnetic Resonance Imaging in MS (MAGNIMS) network experts in 2016 [6]. Expanded Disability Status Scale (EDSS)

is used in disease monitoring in addition to MAGNIMS criteria [7].

Vitamin D has a significant impact on the immune system, demonstrating immunomodulating effects including promotion of T cell differentiation into T-regulatory cells, which produce cytokines, such as TGF-beta (TGF- β). Vitamin D shortage during developmental periods may promote onset of auto-immune diseases, including MS [8].

There is some controversy regarding the role of vitamin D in clinical and laboratory practice with respect to MS disease. Studies to date suggest that vitamin D levels among MS patients may correlate strongly with MRI abnormalities and that vitamin D supplementation may benefit individuals who are predisposed or already suffering from MS.

OBJECTIVE

The aim of this review is to provide an update of existing evidence regarding the link between vitamin D and MRI abnormalities in MS patients. Sources derived from PubMed and Scopus databases spanning the last 10 years of research written in English and relating to vitamin D and MS related MRI abnormalities. Of 85 PubMed and 93 Scopus

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publications consulted, a total of 42 compatible research publications was identified and used to compile this review.

STATE OF KNOWLEDGE

Correlation between vitamin D and immunology of MS.

Vitamin D can affect both innate and acquired immune responses via immunomodulation of antigen presenting cells (APCs), such as dendritic cells, which participate in T cell auto-aggression reduction [9–11]. Moreover, vitamin D plays a vital role in suppressing B and T lymphocyte differentiation [12]. According to recent literature, dysregulation of dendritic cells (the most prolific type of APC) has a significant impact on the pathomechanism of MS. As dendritic cells migrate across the blood-brain barrier, they induce the differentiation of memory T cells into pro-inflammatory T helper 1 (Th1) and T helper 17 (Th17) lymphocytes [13]. The relationship between vitamin D supplementation and alteration of immunological responses has previously been assessed. Patients were split to 3 groups, which received 0 IU (placebo), 5,000 IU and 10,000 IU of vitamin D supplementation per day. Blood samples collected at 16 and 24 weeks of supplementation were analyzed for interleukin 17 (IL-17) or interferon gamma (IFN γ) positive, CD4 positive (CD4+) T cell subsets and cytokine (IL-17, IL-10 and IFN γ) levels. No significant reduction in either IFN γ +CD4+ or IL-17+CD4+ T cells was observed, and no significant differences were observed in concentrations of IL-10, IL-17 and IFN γ . According to the above-mentioned study, vitamin D supplementation did not have an impact on immune system functions [14]. The impacts of vitamin D on the immune system and the mechanisms by which it regulates immune cell differentiation, cytokine secretion and modulates T cell auto-aggression are not yet thoroughly understood, but may have therapeutic implications.

Vitamin D and MS activity in MRI. Increasing evidence suggests that abnormally low levels of vitamin D may be associated with increased MS occurrence and relapses. Blood Vitamin D levels may be an important indicator preceding MRI-detectable abnormalities in the onset of MS. Vitamin D serum concentrations in MS patients may therefore be a crucial and early predictor of the disease. Mowry et al. analyzed the relationship between vitamin D blood level and risk of developing new MRI abnormalities. Observation over 5 years involving annual MRI scans showed that each surge of 25 nmol/L vitamin D blood level resulted in a 15% reduced risk of developing new lesions, monitored by T2-weighted MRI, and a 32% reduced risk using gadolinium-enhanced (Gd+) MRI. Lower serum concentrations of vitamin D were associated with more rapid disease progression [15]. Similar results were reported by Fahmi et al., who noted a significant inverse correlation between blood vitamin D levels and MS lesion load ($p < 0.01$) [16].

In a cohort study involving 88 untreated patients with relapsing-remitting MS, seasonal variations in vitamin D levels were monitored in combination with MRI examinations. Patients experiencing seasonal fluctuations in vitamin D levels exhibited an increased frequency of changes in Gd+ T1 MRI scans (53% of patients) than patients who lacked signs of fluctuating vitamin D levels (23% of patients) ($p = 0.004$) [17]. Recently, Bäcker-Koduah et al. reported that lower serum

vitamin D levels were associated with an increased number of changes in T2-weighted MRI scans. However, the authors did not correlate low levels of vitamin D with a decline in grey matter, white matter and total brain volumes [18]. In other studies, no association between vitamin D levels and brain volume was observed [19, 20]. Wawrzyniak et al. reported significant progression of new changes in T2-weighted MRI scans among patients with vitamin D levels below 20 ng/ml ($p < 0.05$) [21]. In a study by Abbateemarco et al. involving 130 patients who suffered from primary progressive MS (PPMS) and 137 patients with secondary progressive MS (SPMS), no significant associations between levels of vitamin D and T1/2-weighted MRI lesion volume and brain parenchymal fraction were observed. However, correlations between calcifediol (25-hydroxyvitamin D, 25(OH)D) levels and the magnetization transfer ratio (MTR) of whole brain (WB-MTR, $p = 0.007$) and of normal appearing gray matter (NAGM-MTR; $p = 0.02$) have been determined, which may indicate a protective role of vitamin D towards myelin [22].

To investigate the mechanisms by which vitamin D affects MRI-detectable myelination, Aivo et al. evaluated the relationship between vitamin D and cytokines levels. The authors observed that MS patients under IFN β -1b treatment and who also received 20,000 IU of cholecalciferol weekly, had elevated levels of TGF- β which increased, on average, from 47 pg/ml to 55 pg/ml ($p = 0.0249$) after 12 months ($p = 0.0249$). Notably, there was no significant difference in the levels of other cytokines and levels of IFN γ , IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6/IL-13, IL-4, IL-5, IL-1 β , and TNF- α remained mostly unchanged [23]. These findings would suggest an immunomodulating effect of TGF- β which may be contributing to improvements in MRI-detectable myelination MS patients.

The majority of the studies described above concluded that higher vitamin D levels are associated with lower prevalence of MRI abnormalities and slower disease progression in MS patients. It has been suggested that an increase in TGF- β levels could be responsible for the neuroprotective character of vitamin D.

Seasonality and exacerbations of MS in MRI. The possible connection between vitamin D levels and increases in MRI abnormalities suggests a possible link to seasonal variations in sun exposure. Sistani et al. assessed demyelinating lesions in cervical spine and brain MRI scans, and related these to cholecalciferol concentrations in 90 MS patients who had never been on vitamin D supplementation before and who were selected based on McDonald's criteria. Blood vitamin D levels were monitored over the course of one year and average levels were found to be much lower in winter (33.58 nmol/L) than in autumn (214.41 nmol/L) ($p = 0.018$). Among the 90 patients taking part in the study, the number of relapses in spring, summer, autumn and winter were as follows: 26 (28.88%), 15 (16.66%), 32 (35.55%) and 17 (18.89%). A significant correlation between vitamin D serum concentration and the number of demyelinating plaques in cervical spine was observed ($p = 0.007$), whereas no such correlation was found with respect to brain tissue ($p = 0.32$). The authors were therefore cautious in suggesting a connection between seasonality and incidence of relapses [24].

In a study involving 264 patients diagnosed with MS (mean age 46) and 69 people without MS, Zivadinov et al. observed that patients who had more extensive sun exposure

during summer exhibited a higher volume of grey matter and higher overall brain volume than those experiencing less sun exposure. However, their vitamin D blood levels did not correlate with MRI abnormalities, and the connection between sun exposure, vitamin D and MRI-detectable demyelination remains inconclusive [25]. The aforementioned results suggest that vitamin D supplementation may have a greater impact than sun exposure on MS disease progression

Vitamin D supplementation and MS progression. Camu et al. divided patients with relapsing-remitting multiple sclerosis (RRMS) into a group receiving cholecalciferol supplementation and another control (placebo) group. Patients receiving vitamin D supplementation had a significantly lower incidence of new changes in T1-weighted images, as well as a decrease in the volume of pre-existing lesions ($p=0.03$). No difference was observed in either the number of lesions or in grey and white matter volume by Gd⁺-enhanced, T1- and T2-weighted scans [26].

Another study investigated the correlation between vitamin D levels and the progression of retrobulbar neuritis to MS. During a 12-month observation period, none of the patients receiving vitamin D supplementation developed MS, whereas 5 individuals from the placebo group (who did not receive vitamin D supplementation) did develop MS ($p=0.007$). No significant myelin abnormalities were observed by MRI at the start of the study. During the period of observation, subjects receiving supplementation exhibited far fewer MRI changes than the control group, suggesting vitamin D may inhibit disease progression [27].

The effects of excess vitamin D supplementation remain even more uncertain. Stein et al. differentially supplemented patients with RRMS with either high doses (6,000 IU) or small dosage (1,000 IU) of vitamin D. MRI scans were taken at 4.5 and 6 months after supplementation was started. No significant differences in MRI-based signs of MS were observed between the 2 groups [28]. Smolders et al. investigated the impact of vitamin D supplementation (14,000 IU per day) on neurofilament light chains (NfL) levels. NfL protein levels are a biomarker of MS onset. After 48 weeks of supplementation, patients who received vitamin D supplementation did exhibit higher vitamin D serum levels compared with the control group ($p<0.01$); however, NfL levels did not significantly differ between the groups ($p=0.74$). An increase in NfL levels at 48 weeks was associated with a higher risk of disease progression, but vitamin D did not appear to affect disease severity in this study [29]. Similarly, no correlation between natural (un-supplemented) vitamin D levels and NfL ($p=0.95$) were reported by another study which monitored participants over a period of 24 months [30].

Contrary to the above-mentioned findings, a further study reported that high serum levels of vitamin D, in this case 75 nmol/L or higher ($p=0.021$) and 100 nmol/L or higher ($p=0.016$), were associated with lower levels of NfL in cerebrospinal fluid. NfL levels also correlated with the number of contrast-enhancing brain lesions on MRI ($p<0.0005$) [31]. Furthermore, in a study using the EDSS to described the relationship between vitamin D serum levels and disease severity, patients with MS featured significantly lower vitamin D levels than healthy volunteers (15.9 nmol/L vs. 20.6 nmol/L; $p<0.0001$). There was a negative correlation between participants' vitamin D serum levels and their EDSS

score ($p<0.0001$). Serum concentrations below 50 nmol/L indicated higher risk of MS occurrence ($p=0.024$) and an increased likelihood of disease progression ($p<0.001$) [32]. In addition, little difference in EDSS score was observed between 2 groups of MS patients receiving either 20,400 IU or 400 IU over a period of 18 months ($p=0.64$), suggesting that vitamin D in excess doses does not have a differential impact on MS progression [33].

In summary, although some studies did suggest that vitamin D can slow MS progression and halt conversion from retrobulbar neuritis to MS, most of the cited studies could not conclusively confirm a correlation between MS activity, NfL levels and vitamin D blood concentration

Vitamin D and Clinically Isolated Syndrome. Clinically isolated syndrome (CIS) is considered to be one of the first indicators of MS clinical syndrome and associated with a high risk of developing MS particularly when lesions are detectable by MRI [34]. A correlation between vitamin D level and the likelihood of CIS progression towards MS has been suggested. Shaheen et al. monitored the vitamin D serum concentrations of 43 patients with CIS over the course of one year to assess the relationship between vitamin D levels and changes in MRI abnormalities. It was noticed that lower vitamin D levels were more frequently determined in patients who progressed from CIS to clinically definite multiple sclerosis (CDMS), compared with patients who did not progress ($p<0.001$). The authors also reported an inverse correlation between vitamin D blood levels and MRI lesion load seen in T2-weighted scans ($p=0.01$), further suggesting possible benefits of vitamin D supplementation in patients exhibiting CIS [35].

A higher risk of MS onset among patients exhibiting both CIS and low levels of vitamin D was suggested by Martinelli et al. in a retrospective study involving 100 patients with CIS; the authors assessed the effects of vitamin D blood levels on progression to CDMS. Higher risk of CDMS was observed among patients with very low (mean 3.34 nmol/L, range 1.32–8.45) and low (mean 2.04 nmol/L, range 0.96–4.36) vitamin D serum concentration, representing below 10% and below 25% of normal levels, respectively [36]. In contrast, Mowry et al., upon examining 65 patients with CIS by MRI observed an increase of 7.8 ml in grey matter volume among patients with vitamin D concentrations exceeding 25 nmol/L ($p=0.025$). Moreover, an inverse correlation between mean level of vitamin D and appearance of new lesions detected by T2-weighted brain MRI was observed ($p=0.096$) [37]. Naeini et al. reported interesting results while studying 30 patients with CIS alongside a control group. They found no correlation between levels of vitamin D and whether patients exhibited CIS ($p<0.05$). However, they did report a significant correlation between vitamin D blood levels and MRI abnormalities ($p<0.05$), which did suggest a link between low vitamin D levels and CIS progression to MS [38].

A randomized trial by Ascherio et al. revealed that in patients who presented with CIS, within 12 months after diagnosis mean serum concentration of vitamin D was a strong predictor of MS onset and progression during the subsequent 4 years. Higher 25(OH)D levels were associated with reduced lesion accumulation in T2-weighted MRI: the relative decrease in number of lesions associated with 25(OH)D increase of 50 nmol/L was 20% / year ($p=0.00002$). Notably, patients participating in the study were qualified

to receive interferon beta-1b (IFN β -1b) treatment. These findings suggest an anti-inflammatory effect of vitamin D and its active metabolite 25(OH)D in high doses [39].

In summary, the afore-mentioned studies suggest a connection between lower vitamin D levels and a higher risk of CIS progression to MS, which may again be attributed to the neuroprotective capabilities of vitamin D.

Influence of vitamin D supplementation on the course of MS treatment. Potential benefits of vitamin D supplementation in MS patients have been strongly advocated, but the possible interactions of vitamin D with MS treatments have not been thoroughly characterized. Anti-CD20 monoclonal antibodies are widely used to treat various conditions and they have been found to have positive therapeutic effects in patients with relapsing-remitting MS. These therapeutic antibodies bind to B lymphocytes and eliminate them from the circulation, which reduces the inflammatory response. Cholecalciferol concentration also highly correlates with inflammation. Linden et al. found vitamin D supplementation during rituximab treatment to reduce inflammation and lower the risk of relapse. In this study, 272 MS patients were divided into 3 groups: one group was prescribed 2,000 IU of vitamin D per day, the second group received about 2,000 IU of over-the-counter vitamin D supplementation per day, and the third group received no vitamin D supplementation (they received placebo). Inflammation was measured indirectly via C-reactive protein (CRP) blood levels, revealing a significant inverse correlation between CRP levels and 25(OH)D levels in the blood ($p=0.042$) [40].

Another randomized study conducted by Hupperts et al. assessed the safety and effectiveness of high doses of vitamin D in 232 patients with relapsing-remitting MS. Patients received 44 μ g IFN β -1a 3 times per week for a period of 3–18 months prior to the study. The patients received high doses of vitamin D (6,670 IU/day) for 4 weeks, followed by 14,007 IU/day for 44 weeks. The placebo group received no supplementation. Results of the study did not conclusively indicate whether vitamin D high dosage supplementation may induce significant positive biological effects during treatment, as the percentage of relapses over 48 weeks was similar in both groups (78.8% and 75.0% respectively). However, high doses of vitamin D were associated with lower mean percentage of changes in MRI abnormalities in 48 weeks with respect to the starting value (3.57% vs. 6.07%). The authors emphasized that changes in MRI scans may be potentially significant, but the study should be extended to a larger sample size or should include patients with more advanced MS [41].

Hongell et al. investigated the impact of vitamin D supplementation on the appearance of new lesions and on lesion enlargement detected by MRI, in patients enrolled in 3rd phase clinical studies of fingolimod (FTY) treatment at 12 and 24 month of therapy. The authors reported that patients who received daily vitamin D supplementation exhibited significantly fewer new changes in MRI scans, both at 12 and 24 months of observation ($p=0.038$ and $p=0.009$). No differences were observed between patients who took vitamin D sporadically and those who received no supplementation at all [42]. Similar results were obtained in another study involving patients receiving FTY treatment over a 2-year period [43]. At the beginning of the observation period, patients with normal vitamin D levels (>100 nmol/L) had far

fewer lesions detected by Gd⁺-enhanced and T2-weighted MRI in comparison to patients with vitamin D deficiency (<25 nmol/L). A similar tendency was observed after one and 2 years: in patients with normal vitamin D levels, new lesions appeared less frequently and there was a lower likelihood of enlargement of existing lesions. However, no correlation was determined between vitamin D concentration and the time to first relapse [43].

Fitzgerald et al. investigated the impact of serum vitamin D levels (without supplementation) on MS onset and progression in 1,482 patients being treated with IFN β -1b. Baseline levels of vitamin D were inversely associated with T2-weighted hyperintense lesion volume. Patients with vitamin D serum concentrations above 50 nmol/L had significantly smaller lesions in T2-weighted scans ($p=0.02$), as well as in T1-weighted scans ($p=0.03$). Blood levels of vitamin D were also inversely associated with the appearance of new, active lesions (using T1- and T2-weighted scans) between 0–12 months ($p=0.03$). Vitamin D levels above 50 nmol/L were associated with a 31% lower rate of new MRI-detected lesions ($p=0.001$). Moreover, patients with serum vitamin D levels above 100.0 nmol/L had a 47% lower rate of new active lesions compared with patients who had serum levels of 50.0 to 74.9 nmol/L ($p=0.002$). Levels of vitamin D above 100 nmol/L were not correlated with significant therapeutic effects ($p=0.39$). No significant correlation was noticed in an analysis between baseline vitamin D levels and the number of relapses in the previous 2 years ($p=0.77$), or between vitamin D levels and the number of relapses from start of observation to the last visit ($p=0.92$). Vitamin D serum concentrations above 50 nmol/L were initially associated with mean 0.28 lower EDSS scores ($p<0.001$). However, after longitudinal analyses adjusted for certain clinical features, vitamin D levels were not associated with significant reductions in EDSS score [44].

Therapeutically beneficial synergistic interaction between vitamin D and IFN β has been reported with respect to their immunomodulatory effects. Feng X. et al. confirmed that vitamin D supplementation over time, together with IFN β treatment, decreased the level of pro-inflammatory Th1 and Th17 cytokines, but increased the serum concentration of anti-inflammatory Th2 cytokines (IL-4, IL-5 and IL-10). The induction was observed of antiviral myxovirus resistance protein A (MxA) and adhesion molecules molecules (which are indicators of INF response) ved. The authors asserted that vitamin D modifies IFN responses in both lymphocytes and monocytes, inducing changes in cytokine production from Th1 and Th17 to Th2, as well as increasing the number of CD4 regulatory T cells [45].

The effects of vitamin D in MS patients treated with IFN β -1b were examined in the multicentre BENEFIT study, which involved 18 countries and 98 centres, over a period of 2 years. Disease progression was assessed by the number of MRI lesions using Gd⁺ contrast. An extensive analysis of gene expression was carried out to characterize the mechanism of vitamin D's therapeutic and immunomodulatory effects. Expression profiles were determined at various time intervals to investigate the potential relationship between genes or gene sets expressed in relation to 25(OH)D and MS progression. Increased expression of 25(OH)D related genes was associated with fewer lesions in Gd⁺ scans, independently of IFN β -1b treatment. Hence, 25(OH)D may act synergistically with IFN β -1b to inhibit the formation of new visible lesions [46].

The effects of vitamin D on MRI abnormalities were investigated by Rotstein et al. in a study including 324 patients with relapsing-remitting MS, of whom 151 were treated with glatiramer acetate (GA), 96 were treated with IFN β , and 11 received treatment with FTY. The study monitored the time to relapse or new lesion appearance using Gd+ T1-weighted MRI scans after 25(OH)D administration. Patients receiving IFN β in combination with 25(OH)D administration had the greatest reduction in risk (59%) of developing new active MRI lesions, compared with a 43% decrease patients treated with GA, and an approximately 50% decrease in new inflammatory episodes and relapses in patients treated with FTY. The immunomodulatory effects of vitamin D can be explained by the different immunological mechanisms of each of the therapies used, characterized by distinct effects on T lymphocytes. However, the authors emphasized that further studies with larger patient cohorts are needed to verify these results [47].

In summary, the above-mentioned studies indicate that vitamin D may have synergistic effects with ongoing IFN β -1b treatment, reducing the number and the size of newly-appearing lesions in MRI examinations.

CONCLUSIONS

An increase in vitamin D blood levels probably stimulates a surge in TGF- β levels, which could alter existing lesions via its immunomodulating properties. Further research is needed to determine the effect of cholecalciferol on brain MRI lesion load. The potential benefits of vitamin D supplementation among patients with CIS, warrant further investigation. Combining vitamin D supplementation with FTY or IFN β -1a treatment may improve treatment outcome and may even reduce the number of existing lesions observed in brain MRI scans. These findings advocate for the maintenance and sustainment of ideal vitamin D serum concentrations, as this may lower the risk of developing MRI-detected abnormalities related to demyelination.

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