ORIGINAL ARTICLE

Vitamin D in clinically isolated syndrome: evidence for possible neuroprotection

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Abstract

Background and purpose: Vitamin D status has been associated with inflammatory activity in multiple sclerosis (MS), but it is not known if it is associated with gray matter volume, the loss of which predicts long-term disability in MS. The association of vitamin D levels with brain volume measures and inflammatory activity in patients with clinically isolated syndrome (CIS) was investigated.

Methods: In the phase 2 CIS trial of atorvastatin, 25-hydroxyvitamin D levels were evaluated for their age-adjusted associations with normalized gray matter and brain parenchymal volumes on brain magnetic resonance imaging (MRI). The relationships between 25-hydroxyvitamin D levels and clinical and MRI measures of inflammatory activity were also assessed.

Results: In 65 patients in this substudy, each 25 nmol/l higher 25-hydroxyvitamin D level was associated with 7.8 ml higher gray matter volume (95% confidence interval 1.0, 14.6, $P = 0.025$). There was a tendency for an inverse association of average 25-hydroxyvitamin D levels and the composite end-point of ≥3 new brain T2 lesions or ≥1 relapse within a year (odds ratio per 25 nmol/l higher 25-hydroxyvitamin D level 0.66, 95% confidence interval 0.41, 1.08, $P = 0.096$).

Conclusions: Vitamin D status may impact neurodegeneration after CIS, although these results should be replicated in a second study. If confirmed in clinical trials, vitamin D supplementation may reduce long-term disability.

Introduction

Higher 25-hydroxyvitamin D levels appear to protect against the development of multiple sclerosis (MS) [1]. Additionally, in patients with relapsing–remitting MS or clinically isolated syndrome (CIS), those with higher 25-hydroxyvitamin D levels develop fewer relapses and new lesions on brain magnetic resonance imaging (MRI) [2–4].

In MS patients, 25-hydroxyvitamin D_3 levels negatively correlated with low density lipoprotein levels and with the total cholesterol to high density lipoprotein ratio, suggesting a possible interplay between vitamin D and aspects of cholesterol metabolism [5]. In this investigation, the STAyCIS trial, a randomized trial of atorvastatin (a cholesterol-lowering medication) versus placebo in patients with recent CIS, was used to assess the association of 25-hydroxyvitamin D levels with imaging measures thought to reflect neurodegeneration. Of special interest was gray matter volume because shorter-term reductions thereof seem to reflect neurodegeneration, eventual disability in MS is believed to be due to neurodegeneration [6], and shorter-term reductions in gray matter volume are associated with longer-term disability [7–20].

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Methods

Subjects enrolled in STAyCIS (NCT00094172) were included if they had stored serum from the baseline or month 6 study visit and had at least one subsequent

follow-up visit [20]. STAyCIS was an institutional review board approved, randomized, double-blind, multicenter, placebo-controlled phase 2 trial of oral atorvastatin 80 mg daily versus placebo (3:2 block randomization, stratified by center) in treatment-naïve subjects with CIS. Full eligibility criteria are described in the original paper [20]. The trial was stopped due to slow recruitment after 82 subjects (of 152 planned) had enrolled.

STAyCIS subjects were evaluated clinically and by brain MRI at baseline and at months 3, 6, 9 and 12; additional clinical visits occurred at months 1 and 2. The primary end-point for the trial was the development of ≥1 MS relapse or ≥3 new T2 lesions on brain MRI at month 12 [20]. This end-point will be referred to as the 'STAyCIS composite inflammatory end-point' to distinguish that it was not the main focus of the current study. Patients who met this end-point during the trial and completed at least 6 months of the study were eligible to start weekly intramuscular interferon beta-1a. Patients who did not meet this end-point and had no new brain MRI lesions after month 3 discontinued study medication after month 12 but had additional clinical visits and repeat brain MRIs at months 15 and 18. The remainder of the patients discontinued study medication after month 12 and had clinical visits, but not MRI scans, at months 15 and 18.

Brain MRIs were performed using a standardized protocol, and lesion and normalized brain volumes were blindly analyzed, as previously described [20]. Steroids were not given within 28 days of MRIs.

From stored sera at baseline and at month 6, total 25-hydroxyvitamin D levels were measured in one single batch by chemiluminescent immunoassay (Diasorin Liaison, Heartlands Assays Inc., Ames, IA, USA). Levels are presented in nmol/l (1 ng/ml = 2.496 nmol/l). The intra-assay coefficient of variation is 8.1%. Because seasonal adjustment did not meaningfully affect our previous analyses and since they are more interpretable, the levels were not adjusted for season [4].

Repeated measures analyses (generalized estimating equations with robust standard errors, assuming a Gaussian distribution) were used when normalized gray matter volume (nGMV) and brain parenchymal volume (nBPV) were the outcomes, and MRI scans from month 12 and month 18 were used (for two patients missing month 12 MRI but who had a month 15 scan, the latter was used in the place of the former). Poisson generalized estimating equations with robust standard errors and an offset of the log time since the prior MRI were used when the outcome was the number of new gadolinium-enhanced lesions or the number of new T2-weighted lesions. An exchangeable correlation matrix was employed for all repeated measures models. For all lesional MRI outcomes, analyses were restricted to the first 12 months in the study because patients only had MRIs after the month 12 visit if they had not met the STAyCIS composite inflammatory end-point; since the development of new lesions contributes to that end-point, including the scans done after month 12 may have introduced bias. For the risk of a second clinical event over the entire duration of follow-up, a Cox proportional hazards model was used. Logistic regression models were used to explore the odds of meeting the STAyCIS composite inflammatory end-point within the first year.

The primary predictor in all models (except for meeting the STAyCIS composite inflammatory end-point) was the prior 25-hydroxyvitamin D level, evaluated in 25 nmol/l (rounded from 24.9 nmol/l for readability) increments. The baseline 25-hydroxyvitamin D level was used as the primary predictor for outcomes up to and including month 6; the month 6 25-hydroxyvitamin D level was used for subsequent outcomes. When the month 6 level was missing, the baseline 25-hydroxyvitamin D level was used as a proxy, whereas the month 6 level was never used as a proxy for the baseline level. When meeting the STAyCIS composite inflammatory end-point was the outcome, the average of 25-hydroxyvitamin D levels at baseline and month 6 (when both were available) was the primary predictor. In addition to adjusting models for age, whether adding sex, race (Caucasian versus non-Caucasian), baseline body mass index (BMI, kg/m^2), baseline disability (on the Expanded Disability Status Scale) or treatment group (atorvastatin versus placebo) influenced the results was explored. For brain volume outcomes, gadolinium-enhancing lesions or new T2-weighted lesions on the concomitant scan were also evaluated as covariates. No patient who had an attack during the study initiated disease-modifying therapy prior to the attack, and only two patients began therapy prior to developing ≥3 new T2-weighted lesions, so use of interferon therapy (considered 'on' when the patient received at least 3 months of therapy to account for the delay in onset of its effect) was assessed for its influence on the results only when the outcome was a pure MRI outcome. To explore whether the relationship between 25-hydroxyvitamin D and a given outcome differed depending on atorvastatin assignment, an interaction term between 25-hydroxyvitamin D levels and treatment group was generated and evaluated in models in which brain volume and the STAyCIS composite inflammatory end-point were the outcomes. When the

P value for the interaction term was <0.1, the association of 25-hydroxyvitamin D with the outcome in each treatment group was evaluated.

Results

Of 82 patients in the trial, 65 were eligible for inclusion in this substudy. 25-hydroxyvitamin D levels were missing for only three at baseline and two at month 6; brain volume segmentation could not be performed for eight patients. Demographics are presented in Table 1.

More than half of the patients (n = 36, 55%) met the STAyCIS composite inflammatory end-point; 19 (29%) had a second attack during the entire follow-up period (15 in the first 12 months). The median number of new T2 lesions on a given scan was 0 (interquartile range 0, 6), and the median number of gadolinium-enhancing T1-weighted lesions was also 0 (interquartile range 0, 3) at month 12 or month 18. Whilst those in the atorvastatin group had slightly higher baseline 25-hydroxyvitamin D levels than those in the placebo group [13.3 nmol/l higher, 95% confidence interval (CI) −1.8, 28.3, P = 0.082], adjusting models for treatment group did not appear to modify the results in any of the analyses.

There appeared to be an association between 25-hydroxyvitamin D levels and nGMV (Table 2); for every 25 nmol/l higher 25-hydroxyvitamin D level, the nGMV was 7.8 ml higher (95% CI 1.0, 14.6, P = 0.025). The results were only mildly attenuated when adjusted for race (6.0 ml, 95% CI −0.9, 12.9, P = 0.088) or BMI (6.5 ml, 95% CI −2.2, 15.2, P = 0.14), but not when other potential confounders were added. There was no apparent interaction between 25-hydroxyvitamin D and the atorvastatin group (P = 0.72). The results were similar when only the 12-month MRI was evaluated, although the confidence intervals were wider. The repeated measures models for nBPV were too unstable to provide reliable estimates of association, so multivariate regression models using month 12 nBPV was used for this outcome. Each 25 nmol/l higher 25-hydroxyvitamin D was associated with 2.9 ml higher nBPV (95% CI −11.1, 16.9, P = 0.68). The results were similar when potential confounders were added, and there was no apparent interaction between 25-hydroxyvitamin D levels and treatment group (P = 0.51).

Higher average 25-hydroxyvitamin D level showed some evidence of a trend for an association with a lower odds ratio (OR) for meeting the STAyCIS composite inflammatory end-point (OR per 25 nmol/l, 0.66; 95% CI 0.41, 1.08; P = 0.096) (Table 3). The results were not meaningfully different when the other covariates were added, although the 95% CI was wider when BMI was added (OR = 0.76, 95% CI 0.45, 1.30, P = 0.32). The P value for the interaction term between 25-hydroxyvitamin D levels and treatment group was 0.049: 25-hydroxyvitamin D levels (per 25 nmol/l) were inversely associated with the outcome in the atorvastatin group (OR = 0.46, 95% CI 0.23, 0.93, P = 0.030) but were not apparently associ-

Table 2 Age-adjusted association of vitamin D and brain volume

Outcome	Per 25 nmol/l higher vitamin D level (age-adjusted)
Normalized gray matter volume (ml)	
Overall (repeated measures model)[a]	7.8 (1.0, 14.6), P = 0.025
At month 12 (regression model)	7.9 (−1.6, 17.3), P = 0.10
Normalized brain parenchymal volume (ml)	
Overall (repeated measures model)[a]	Unable to assess
At month 12 (regression model)	2.9 (−11.1, 16.9), P = 0.68

[a]Incorporates brain volume measures using data collected from month 12 and month 18 MRI; for two patients missing month 12 MRI who did have month 15 MRI, the latter time point was used in place of the month 12 image.

Table 1 Baseline characteristics of 65 STAyCIS patients included in the vitamin D substudy

Variable	Placebo (n = 26)	Atorvastatin (n = 39)	P value
Age in years, mean ± SD	34 ± 9	35 ± 9	0.85
Female sex, n (%)	18 (69)	29 (74)	0.65
Expanded Disability Status Scale score, median (interquartile range)	1.5 (1, 3)	1.5 (0, 2.5)	0.84
Body mass index (kg/m^2), mean ± SD	28 ± 6	26 ± 6	0.41
White race, n (%)	23 (88)	36 (92)	0.60
Hispanic ethnicity, n (%)	2 (8)	1 (3)	0.33
Normalized brain parenchymal volume (ml), mean ± SD	1680 ± 73	1674 ± 86	0.77
Normalized gray matter volume (ml), mean ± SD	983 ± 65	987 ± 62	0.81
T2-weighted lesions, median (interquartile range)	16.5 (5, 36)	18 (3, 44)	0.93
Gadolinium-enhancing T1-weighted lesions, median (interquartile range)	0 (0, 1)	0 (0, 1)	0.79
25-hydroxyvitamin D level (nmol/l), mean ± SD	56 ± 21	70 ± 33	0.082

Table 3 Age-adjusted association of vitamin D and inflammatory outcomes

Outcome	Age-adjusted model estimate of risk or odds (per 25 nmol/l higher vitamin D level)
STAyCIS composite inflammatory end-point (by month 12)[a]	0.66 (0.41, 1.08), $P = 0.096$
T2-weighted lesions[b]	0.89 (0.69, 1.14), $P = 0.35$
Gadolinium-enhancing lesions[b]	0.95 (0.65, 1.37), $P = 0.77$
Clinical attack within entire follow-up period[c]	0.92 (0.63, 1.35), $P = 0.68$

[a]Odds ratios, [b]incidence rate ratios or [c]hazard ratios given with 95% confidence intervals and *P* values.

ated with the outcome in the placebo group (OR = 1.61, 95% CI 0.57, 4.55, $P = 0.37$).

When the entire duration of follow-up was considered, 25-hydroxyvitamin D levels did not appear to be meaningfully associated with risk of a second clinical attack (hazard ratio per 25 nmol/l greater 25-hydroxyvitamin D, 0.92; 95% CI 0.63, 1.35, $P = 0.68$) regardless of the covariates added. 25-hydroxyvitamin D levels also did not appear to be meaningfully associated with the number of new T2-weighted lesions on brain MRI (incidence rate ratio per 25 nmol/l higher 25-hydroxyvitamin D level, 0.89; 95% CI 0.69, 1.14, $P = 0.35$) or gadolinium-enhancing lesions (incidence rate ratio per 25 nmol/l higher 25-hydroxyvitamin D, 0.95; 95% CI 0.65, 1.37, $P = 0.77$). The results were not substantially changed when putative confounders were considered.

Discussion

In this study, there was an age-independent association of 25-hydroxyvitamin D levels with preserved nGMV in patients with early CIS. Adjustment for new inflammatory activity on MRI and other potential confounders, including atorvastatin treatment, did not attenuate the relationship. Since preserved nGMV appears to be associated with reduced long-term disability in MS, these results, if confirmed in larger studies and then clinical trials, have enormous implications for treatment of MS patients, especially since the 7.8 ml higher brain volume associated with just a 25 nmol/l higher vitamin D level represents 0.79% of the mean baseline nGMV and a longitudinal study showed that 0.44% loss of nGMV each year was associated with clinical worsening [11]. If supplementation with vitamin D does preserve gray matter with a similar degree of magnitude over the same time frame, then increasing a person's 25-hydroxyvitamin D levels by 87 nmol/l, a feasible goal, would be associated with nearly 3% less reduction in gray matter volume over 18 months. Such an effect size would suggest that vitamin D supplementation would be quite impactful in reducing clinical disability in MS patients.

This is the first investigation to report such an association. One study showed the ratio of 25-hydroxyvitamin D_3 to 24,25-dihydroxyvitamin D_3 was inversely correlated with nBPV (partial $r = -0.21$), but the strength of the correlation was weak, and the study was cross-sectional [21]. Another study reported a trend ($P = 0.07$) for an association of higher 25-hydroxyvitamin D levels with preserved whole brain volume but did not assess gray matter volume separately [22]. A meaningful relationship between 25-hydroxyvitamin D status and nBPV was not found in our study, which it is hypothesized may be related to the short study duration, small sample size, or the apparent stability of white matter volume compared to nGMV over time in MS [11,18].

Known to have neurotrophic properties, vitamin D and its receptor are found throughout the central nervous system [23–25]. Vitamin D is neuroprotective in cell culture models of traumatic brain or spinal cord injury and ischaemia [26–29] Lower vitamin D levels or intake have been implicated in human neurodegenerative disorders such as Alzheimer and Parkinson diseases [30,31] and, in a retrospective study of patients with progressive MS (thought to be due to neurodegeneration rather than inflammation), those who progressed faster reported less use of cod liver oil in childhood and less sun exposure prior to disease onset [32].

Higher levels of 25-hydroxyvitamin D tended to be associated with reduced odds of meeting the STAyCIS composite inflammatory end-point. The associations of 25-hydroxyvitamin D levels with other inflammatory outcomes did not appear to be strong in this study, although in view of the sample size one cannot conclude that a true association of 25-hydroxyvitamin D levels and these outcomes does not exist.

There did appear to be a possible interaction between 25-hydroxyvitamin D levels and atorvastatin when the dependent variable was the STAyCIS composite inflammatory end-point. The finding could be a false association, but some statins, including atorvastatin, are thought to increase vitamin D levels [33], and 1,25-hydroxyvitamin D_3 levels have been shown to predict subsequent biomarker response to atorvastatin treatment [34]. Thus, it may be that there is a true interaction between statin use and vitamin D in modulating inflammatory activity in MS, which deserves further investigation in future, larger studies.

This study has some limitations. The small sample size (and limited occurrence of some outcomes) led to wide confidence intervals for many estimates, such that some associations might have been missed. The smaller number of MRI scans included in multivariate linear regression models in which nGMV at month 12 was the outcome likely accounts for the widened confidence intervals in those models. Ideally, a future study would be longer, with vitamin D measures at frequent time points to refine the estimates, assess whether the associations seen are consistent over time and to explore if 25-hydroxyvitamin D levels are associated with sustained changes in clinical disability. The interaction model findings should be interpreted with caution.

Future studies may consider assessing cholesterol status or statin use to determine if any interaction with vitamin D does exist; they should also carefully record vitamin D supplement use to better evaluate if statins independently influence 25-hydroxyvitamin D levels. Finally, clinical trials are needed to determine if oral vitamin D helps prevent long-term MS-related disability or impacts MRI metrics that reflect neuronal integrity.

Disclosure of conflicts of interest

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