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TO INVESTIGATE THE ASSOCIATION OF DOSE-DEPENDENT VITAMIN D SUPPLEMENT ON CLINICAL OUTCOMES IN VETERANS WITH MULTIPLE SCLEROSIS

Neurology

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ABSTRACT

Objective: To investigate the association of daily dose-dependent Vitamin-D supplement on clinical outcomes including mortality in veterans with multiple sclerosis (MS) with low serum Vitamin-D (25[OH]D) levels. **Methods:** This observational, longitudinal study reports on data collected from 1/1/2000 to 12/31/2020 at the MS Regional Program. We reviewed the electronic records of 163 veterans with MS who were followed every four months in our MS clinic. The study sample was divided into three groups based on the daily Vitamin-D supplement doses: low (1000 IU/day), medium (2000 IU/day) and high (4000 IU/day). The clinical outcomes were changes in physical disability (Expanded Disability Severity Scale (EDSS)), function (Total Function Independence Measures (TFIM)), time-to-event EDSS score of 6 (progressive MS) and mortality. **Results:** There were 32 patients in the low, 46 in the medium, and 85 in the high-dose daily Vitamin-D supplement groups. The mean age of the entire study sample was 51 12 SD years (range 23 - 79 years). The mean duration of MS was 21.7 13 years. The mean initial EDSS and TFIM scores were 3.4 2.8 and 111 17 SD. At entry, mean serum 25[OH]D and calcium levels were 30 16 nmol/l and 9.3 0.4. The risk of MS-related progressive disability (time-to-event EDSS score 6) was lower and there were fewer deaths in the high- (compared to low- and medium-) dose groups. **Conclusion:** This study suggests that among veterans with MS and low 25[OH]D levels, those who received high-doses daily of supplemental Vitamin-D supplement were associated with a decreased risk of MS-related progressive disability and death than those who received a lower dose. No adverse clinical or biochemical events were noted.

KEYWORDS

Serum Vitamin-D level, Cholecalciferol, Disability, Mortality, Veterans, Multiple sclerosis, Observational

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system with a variable course. MS mainly affects young adults, with the highest incidence between the ages of 20 and 40 years. Its etiology is multifactorial, with both genetic susceptibility and environmental factors contributing to its pathogenesis [1]. One environmental factor implicated in the development and progression of MS is the low serum levels of Vitamin-D (25[OH]D). [2] Munger *et al*. prospectively, investigated serum 25[OH]D levels and risk of MS in 7 million US military personnel. He found that high serum 25[OH]D levels were associated with a lower incidence of MS; with 41% lower odds in the *rate* of developing MS for every 50 nmol/L increase in Vitamin-D levels irrespective of the patient's gender. [3] In a 52-week trial of patients with MS, annualized relapse rate (ARR) decreased from 0.44 to 0.26 events in 25 patients with MS placed on calcium and Vitamin-D supplement (with doses as high as 40,000 IU per day) compared to ARR decrease from 0.54 to 0.45 in 24 patients with MS on no supplement a 41% reduction in the number of new MS *events*. [4]

Vitamin D is a fat-soluble vitamin that can be absorbed from food, but its main source is the skin under the influence of ultraviolet (UVB) light. Almost 50% of white and 75% of black adults in the US had 25[OH]D levels below 70 nmol/L, with an overall decline of 9% in mean serum 25[OH]D levels between 1988-1994 to 2001-2006. [5]

Vitamin-D has pleiotropic effects in addition to its well-known musculoskeletal effects. [6] Metabolic effects of Vitamin D include prevention of hyperparathyroidism. Its pleiotropic effect includes anti-inflammatory effects by increasing Th2 anti-inflammatory CD4 lymphocytes thereby affecting the Th1/Th2 ratio. [7]

There is a dramatic increase in the prevalence and incidence of MS with increasing distance from the equator correlated with limited exposure to sunlight and UVB. [8] A decrease in MS development has been documented in those exposed to increased sun exposure in early life. [9] Patients with MS are likely to have low serum 25(OH)D levels particularly in the summer months, because heat intolerance limits the time spent in sunlight. [10] The proposed neuroprotective effects of the active form of 25[OH]D in MS includes its anti-ischemic, antioxidant, and immunomodulatory effects by enhancing regulatory T-cell function, [11] and antimicrobial effects by reducing the risk of respiratory infections, [12] a known trigger of MS relapses. [13]

Our objective was to determine the relationship between low (1000IU/day), medium (2000 IU/day), and high (4000IU/day) daily doses of Vitamin-D supplement on physical disability, functional outcome, and mortality in veterans with MS with low 25[OH]D levels. Our hypothesis was that daily high-dose Vitamin-D supplement would be associated with smaller decreases in physical disability, a longer time for patients to reach an Expanded Disability Severity Scale (EDSS) score of 6 (a cut score considered to have progressive MS), and a lower incidence of death. Daily high-dose vitamin D supplementation is inexpensive, safe, and easy to administer. Confirmation of this hypothesis would support its use in reducing physical impairments and mortality in veterans with MS.

METHODS

Participants

This retrospective, observational study used longitudinally data collected from 163 veterans diagnosed with MS (using the McDonald criteria) [14] and followed in our MS program at the Oklahoma City VA Medical Center. In accordance with the Veterans Health Administration policies, we follow all patients with MS every four months to monitor their response to disease modifying therapies (DMTs). This study reports data collected from 1/1/2000 to 12/31/2020. The OKC VAMC is a MS Regional Program as part of the VA MS Center of Excellence and provides MS specialty care throughout the continuum in the acute, chronic, and long-term stages of the disease. The MS clinic has a structured approach in which all MS patients on initial and yearly clinical evaluations have documentation of the type of DMT they are on, functional outcome measures, neuroimaging, complete blood for lymphocyte count, blood chemistry for renal and liver functions, total serum calcium and vitamin D levels [25(OH)D], and John Cunningham virus (JVC) antibody titers.

In this single-center study, of 273 subjects with MS, 163 veterans had complete electronic records including functional outcome measures. One-hundred and ten veterans were excluded due to incomplete electronic records, especially lack of documentation of both initial and final functional outcome measures and serum calcium and serum 25(OH)D levels (Study flow chart (**Figure 1**)).

This study collected data on demographic and clinical measures (age, gender, race, height, weight) and MS status (age at MS onset, clinical MS subtype (Relapsing-remitting MS (RR), Secondary-progressive

MS (SP), Primary-progressive MS (PP)), [15] and duration of the disease). In addition, the presence of co-morbidities (hypertension, hyperlipidemia, diabetes mellitus, current smoking habit, body mass index (BMI)), and MS related complications (fatigue, depression) were recorded. These comorbidities are the most common causes of disability and death in the general US population. Veteran's initial and recent serum calcium and 25[OH]D levels and Vitamin-D supplement dosage for each veteran were collected.

This study conforms to all STROBE guidelines and reports the required information accordingly (see Supplementary Checklist)". This study was approved by the local Review Board (IRB # 5298) and was exempt from patient consent due to the retrospective review of the electronic medical records.

Outcome Measures

Relationship between Vit D supplementation and changes in clinical outcomes (physical disability (EDSS), and function (Total Function Independence Measures (TFIM)) as well as time-to-EDSS score of 6 (suggests progressive MS) and mortality were studied.

The level of disability was measured using the EDSS, with scores ranging from 0 to 10, with higher scores indicating greater physical disability. [16] The EDSS scale is considered the "gold standard" in measuring MS-related disability in MS clinical trials. The EDSS has fair to substantial inter-rater reliability ($\kappa = 0.32$ to 0.76) with a 2-point change considered a reliable indicator of the patient's response to treatment. [17] An EDSS score of 6 and time-to-event EDSS score of 6 (is considered to indicate limited ambulation ability, being dependent; progressive MS) were used to assess long-term treatment benefit.

Function was measured using the TFIM. [18] The TFIM is a reliable [19] and valid [20] functional assessment widely used in many rehabilitation settings to measure the degree of disability (score ranges from 18 to 126, with higher scores representing less disability).

The EDSS, and TFIM scores were documented by a neurologist who was Board and FIM certified at the VA Medical Center. Deaths were recorded from the patient's death certificates for those who died before 12/31/2020.

Serum 25[OH]D levels were measured during the veteran's initial evaluation at enrollment in the MS program and every 12-months follow-up thereafter. Normal values at our facility are 32-100 ng/ml. Serum calcium level was measured, as vitamin D intake can cause hypercalcemia and nephrolithiasis and affect renal function. [21-23]

Intervention or treatment:

Veterans were prescribed a Vitamin D supplement (cholecalciferol) at varying doses from 1000 IU to 4000 IU/day with the aim of achieving a normal 25[OH]D level (32-100 ng/ml). The daily Vitamin-D supplement dose was defined as "low" (1000 IU/day), "medium" (2000 IU/day) or "high" (4000 IU/day).

Veteran's providers prescribed low-dose (1000 IU/day) vitamin D based on the recommendations of the Institute of Medicine. [24] In this study 1000 IU/day was the lowest vitamin D supplement used in our facility. A medium (2000 IU/day) or high dose (4000 IU/day) was prescribed if either a) the serum 25[OH]D levels were below normal at low dose or b) when seen for the first time by either provider (MHR, KJ).

Veteran's initial and recent serum calcium and 25[OH]D levels were collected. Adverse effects of Vitamin D supplementation particularly hypercalcemia and nephrolithiasis, which are known to affect renal function (urea nitrogen and creatinine levels) were observed. [21-23]

Statistical analysis

1. Descriptive statistics for the three groups defined according to the Vitamin-D supplement dose are expressed as mean standard deviation (SD) or grouped frequencies.
2. Continuous variables were compared between the three groups using pairwise Student's t-test or Wilcoxon rank test depending on the data normality, which was examined using the Shapiro-Wilk normality test. Categorical variables were compared among the three groups using pairwise Chi-square tests or Fisher's exact tests.
3. the log-rank test (Kaplan-Meier plot) Log-rank test (Kaplan-Meier plot) and Cox Proportional Hazard (CoxPH) model were used for time-to-event analysis. The proportional hazard assumption in CoxPH was examined using the R function cox.zph.
4. *post-hoc* analysis was performed among veterans for whom the initial and final values were recorded for serum 25[OH]D. This sub-sample was divided according to its median final serum 25[OH]D concentration of <32 ng/ml vs. ≥32 ng/ml based on our laboratory reference range of 32-100 ng/ml being normal. The group whose values fell below the 32 ng/mL include those with Vitamin D deficiency defined as a serum 25(OH)D level of less than 20 ng/mL (50 nmol/L), or Vitamin D Insufficiency defined as "a serum 25(OH)D level of 21-29 ng/mL (52-72 nmol/L). [25]

For baseline characteristics, results corresponding to p-values lower than 0.05 were described as significant for discussion. Data analyses were conducted using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Table 1 describes the study sample of 163 veterans with MS whose mean age at MS onset in years was 37 12, MS duration in years was 21.713, living with relapsing (n=79, 49%), progressive (n=61, 37%), and other types of MS (n=20, 12%). The study sample consisted mostly of men (n=124, 76%) with a moderate level of MS-disability (EDSS mean score 3.4 2.8 SD) and MS-related physical function (TFIM mean score: 111 17 SD). The three groups defined by the Vitamin D supplement dose differed at baseline for age at entry in the study. Pairwise comparisons of the three groups revealed higher oral intake of DMT in those receiving a medium-dose than in those receiving a low-dose of Vitamin D. Pairwise comparisons revealed that the group receiving low dose supplementation had a lower baseline BMI than those receiving the medium dose ($p< 0.05$). Pairwise comparisons revealed a greater increase in the mean final serum 25[OH]D concentration in the group receiving high-dose supplementation (58 ng/dl) than in those receiving the low-dose (50 ng/dl) and medium-dose (47 ng/dl) ($p< 0.05$).

Table 2 presents the primary outcome measures between the three groups. Pairwise comparisons of groups revealed no statistically significant differences in the changes in scores for MS-related clinical outcomes between the groups. However, those receiving high-dose Vitamin-D supplementation experienced a greater loss of function (mean change in EDSS=1.58) than in those receiving medium dose supplementation (mean change in EDSS=0.66). Given that the high-dose group had a greater change in the EDSS score due in part to the initial lesser severity of the disease; when the three groups were compared for the same initial disease severity based on EDSS score ≤ 4 no significant difference in EDSS change between pairwise group comparisons was found. Though no significant difference in the change to an EDSS score of 6 and time to EDSS score of 6 were present between the 3 groups. On closer inspection there were fewer patients who changed to an EDSS score of 6 (50.6%) and a longer time to reach an EDSS score of 6 (7.7 years) was seen in the daily high-dose group. The time to reach an EDSS score of 6 was significantly associated with the vitamin dose groups, as determined by time-to-event analysis based on the log-rank test ($p=0.02$). The risk to reaching EDSS score of 6 was significantly lower in the high-vitamin dose group compared medium- and low-dose combined groups (log-rank $p=0.006$; **Figure 2**). This time-to-event in the EDSS score 6 favoring the high dose supplement group *persisted* after adjusting for baseline variables known to influence MS-related progression: age at entry, gender, BMI, initial EDSS score and DMT use by CoxPH. The proportional hazard assumption was verified and retained.

There were fewer deaths in the high-dose group (n=6, 7.4%) compared to the low- (n=6, 23%, $p=0.065$) and medium-dose (n=9, 24%, $p=0.024$) groups. Death was significantly associated with age and the initial EDSS score. The average age in patients who died was 62.0±8.4 compared to 48.5±11.2 in surviving patients (t-test p-value 2.5e-7). The median initial EDSS score in patients who died was 7.25 compared to 2 in survived patients (Wilcoxon rank test p-value 1.5e-6). The mean initial EDSS in patients died was 6.5±1.9 compared to 3.2±2.7 in survived patients (t-test p-value 8.1e-8).

Table 3 presents a sensitivity analysis motivated by the concern with the above, all-inclusive, analysis that it includes patients who have taken supplement for only a brief period. Therefore, this study repeated

the analysis with only those patients who had taken their supplements for 5 years or more. Certain differences were observed in the overall analysis. Mortality was lower in the high-dose group than those receiving medium-dose group (p=0.009). The time-to-event for an EDSS score 6 and fewer deaths *persisted* favoring the high groups (p < 0.05,).

In **Table 4** when *post-hoc* analysis was performed on the final serum 25[OH]D level using a threshold of 32 ng/ml, we had a sample size of 59 patients. When the low serum (<32 ng/ml) group was compared to the high serum (≥32 ng/ml) group, no significant change was observed in scores for physical disability, functional outcome (TFIM), change to EDSS score 6 and time to EDSS score 6. There was one death in the high-serum group (n=16) compared to nine in the low-serum group (n=43); this result was not statistically significant (p>0.05), which could be due to the small sample size.

No clinical or biochemical adverse events were observed during the study. The mean final serum calcium concentrations in the high-, low-, and medium-dose groups were similar 9.29, 9.36. and 9.33 mg/dl respectively (p>0.05).

DISCUSSION

The main findings of this retrospective, real-world, observational study conducted on the 20-year longitudinally collected data are as follows: i) 52% of veterans with MS were on daily high-dose vitamin-D supplement; ii) the veteran population studied had a lower level of MS-related disability, higher level of function, and lower death rate; and iii) all group of veterans with low initial serum 25[OH]D levels improved their final serum 25[OH]D levels; however, veterans on a high-dose of Vitamin-D supplement (4000 IU/day) increased their serum levels the most. None of the veterans experienced any adverse clinical or biochemical effects of high doses of Vitamin D supplement, such as hypercalcemia.

Prior to this study, there were few small-scale randomized controlled trials of Vitamin-D supplement. These studies have yielded mixed results with improvements in inflammatory markers and MRI findings but no improvement in MS-related clinical measures, including EDSS. Burton *et al.* [4] in their study of 49 MS patients (25 treatment, 24 controls), randomized to initial Vitamin D doses of up to 40,000 IU/day followed by 10,000 IU/day for 12-weeks then titrated down to 0 IU/day with a 52-week follow-up, showed that high-dose Vitamin-D was safe and showed a persistent decrease in T-cell proliferation compared to controls (who were allowed to take <4000 IU/day of Vitamin-D and calcium supplement if they chose to do so). However, there was no difference in the EDSS scores between the two groups. A 96-week randomized controlled trial by Kapman *et al.* [26] on 68 MS patients on 20,000IU Vitamin-D3 weekly plus 500 mg calcium daily (n=35) or placebo plus 500 mg calcium daily (n=36) showed no beneficial effects on MS-related clinical outcome measures such as annualized relapse rate, EDSS, multiple sclerosis functional composite components, grip strength, and fatigue. Another study by Golan *et al.* [27] of 45 interferon-beta treated patients assigned to either low (800IU, n=21) or high (4000IU, n=24) Vitamin-D daily showed no significant differences in EDSS score, relapse rate, quality of life or serum IL-10. Recently Soilu-Hanninen *et al.*, [28] in a 1-year study of 66 MS patients with Vitamin-D as an add-on treatment to interferon-1b, showed a trend towards reduced disability and improved tandem walk in the Vitamin-D group compared to the control group.

Our study showed that when the three groups were compared for the same initial disease severity based on an EDSS score ≤ 4, no significant difference in EDSS change between pairwise group comparisons was found. However, the time to reach EDSS 6 was significantly associated with the vitamin dose groups based on a time-to-event analysis using the log-rank test. The risk of reaching an EDSS score of 6 was significantly lower in the high- compared to low- and medium-dose groups. Fewer deaths and no adverse effects were observed in the high-dose group. This time-to-event finding in the EDSS score 6 favoring the high dose supplement group persisted even after adjusting for significant baseline variables known to influence MS-related progression: age at entry, gender, BMI, initial EDSS score and DMT use. Death was significantly associated with older age and high initial EDSS score.

The present study had several limitations. First, this study was primarily conducted among veterans who were men (76% men, 80% non-Hispanic white), indicating less generalizability to the general population of patients with MS. Second, the relatively small sample size between the supplement groups reduces the power to detect associations and may bias the result. Third, the observational nature of the study may not be the optimal design to assess the efficacy of treatment as opposed to clinical trials; it still reflects real-world experience in which the assigned dosage groups were not deliberately randomized. Finally, only the initial and final vitamin D levels for each patient were considered, whereas the average annual vitamin D levels over time may have been more representative of the patient's exposure to vitamin D.

Despite these limitations, this retrospective analysis of 20-years of longitudinally collected data, recruitment of all patients in the database with periodic follow-up at 4-, 8-, and 12-month time periods, with no loss and the completeness of the data captured by the standardized MS registry provides a relevant rich dataset to better understand the relationship between dose-dependent Vitamin-D supplement on disability, and deaths in veterans with MS and low serum 25[OH]D levels. This study could form the basis of a randomized controlled trial. In a recent SOLAR study, [29] Hupperts et al. found no benefit for high-dose vitamin D_3 (14,007 IU) as an add-on to IFN-β-1a in achieving the primary outcome of NEDA-3 at 48 weeks but did find a decrease in the number of active new lesions on MRI in patients with RRMS. However, the main limitation of this study was the 48 weeks of the study and an increase in the dropout rate of 10%. In the CHOLINE study [30] Camu et al. found that annualized relapse rate was not achieved at 96 weeks in the interferon beta-1a 44g (SC 3 times per week) plus cholecalciferol 100,000 every other week compared to interferon beta-1a 44g (SC 3 times per week) plus placebo in patients with MS having low serum 25[OH]D levels. There were several reasons why cholecalciferol was not beneficial in this study: i) the small sample size of 126 subjects (61 in the cholecalciferol group, 65 in the placebo group), ii) the comparatively short follow-up period of 96 weeks, and iii) increase drop-out rates of 28.6% in the cholecalciferol and 31.8% in the placebo group (normally accepted is ≤ 10%).

CONCLUSIONS

This study suggests that high-dose daily Vitamin-D supplement was associated with a lower MS-related disability (time-to-event and EDSS score 6) and deaths. High-dose daily supplement use did not increase the risk of adverse clinical or biochemical events in this study.

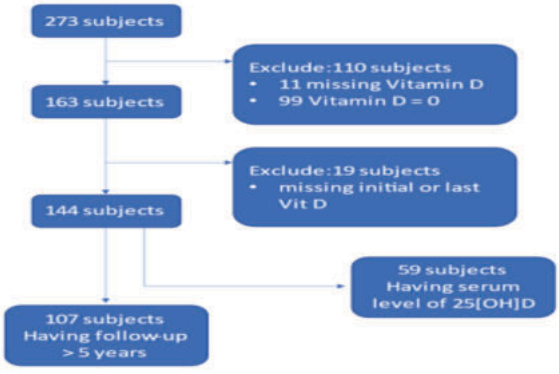

Figure 1. Study Flow Chart

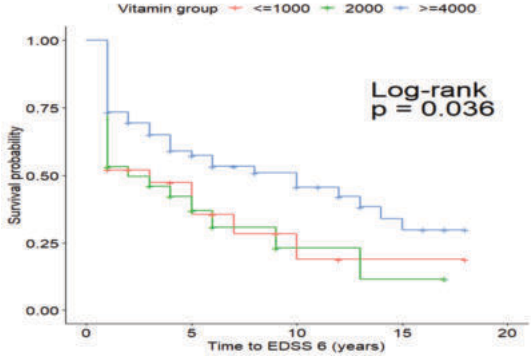

Figure 2. Kaplan-Meier plot of patient's time-to-EDSS 6 by vitamin groups

Table 1. Baseline demographic information and clinical characteristics of veterans with MS prescribed by groups (Mean (SD) or n (%), where applicable)

Grouping Variables (N)	Total Population (N=163)	Vitamin D supplementation			P-value		
		1000 IU/day (N=32)	2000 IU/day (N=46)	4000 IU/day (N=85)	1000 vs. 2000 IU/day	2000 vs. 4000 IU/day	1000 vs. 4000 IU/day
Age @ entry (years)	50.8 (12.1)	55.9 (11.6)	51.9 (13.4)	48.2 (10.9)	0.172	0.109	0.002
Gender							
F	39 (23.9%)	10 (31.2%)	15 (32.6%)	14 (16.5%)	1.000	0.057	0.132
M	124 (76.1%)	22 (68.8%)	31 (67.4%)	71 (83.5%)			
Race*							
American Indian or Alaskan Native	2 (1.2%)	1 (3.1%)	0 (0%)	1 (1.2%)	0.712	0.061	0.230
Black	27 (16.6%)	6 (18.8%)	9 (19.6%)	12 (14.1%)			
Hispanic	4 (2.5%)	1 (3.1%)	3 (6.5%)	0 (0%)			
White	130 (79.8%)	24 (75.0%)	34 (73.9%)	72 (84.7%)			
Age at MS onset (years)	37.4 (12.1)	39.0 (14.3)	38.5 (11.5)	36.1 (11.4)			
MS Duration (n=159) **	21.7 (13.0)	23.9(13.2)	22.4 (14.3)	20.7(12.3)	0.636	0.552	0.276
MS Type*							
I (RR)	79 (48.5%)	15 (46.9%)	19 (41.3%)	45 (52.9%)	0.814	0.455	0.876
II (RP, PP, PR, SP)	61 (37.4%)	12 (37.5%)	20 (43.5%)	29 (34.1%)			
III (CIS, RIS)	20 (12.3%)	4 (12.5%)	7 (15.2%)	9 (10.6%)			
Unclassified category (ADEM)	1 (0.6%)	0 (0%)	0 (0%)	1 (1.2%)			
Missing	2 (1.2%)	1 (3.1%)	0 (0%)	1 (1.2%)			
Initial Expanded Disability Scale Score (n=162) **	3.47 (2.79)	3.55 (2.89)	4.12 (2.81)	3.09 (2.71)	0.371	0.046	0.450
Initial Total Functional Independence Measure Score (n=154) **	111 (17.6)	108 (19.4)	111 (15.1)	113 (18.3)	0.743	0.477	0.265
Hypertension (n=157)	80 (49.1%)	19 (59.4%)	23 (50.0%)	38 (44.7%)	0.458	0.932	0.275
Diabetes Mellitus (n=158)	24 (14.7%)	5 (15.6%)	9 (19.6%)	10 (11.8%)	0.987	0.386	0.763
Hyperlipidemia (n=154)	82 (50.3%)	17 (53.1%)	19 (41.3%)	46 (54.1%)	0.367	0.180	1.000
BMI (n=154)	28.3 (5.62)	26.4 (4.89)	29.6 (6.70)	28.3 (5.06)	0.021	0.255	0.084
Current Smoker (n=157)	59 (36.2%)	12 (37.5%)	17 (37.0%)	30 (35.3%)	1	1	1
Depression	101 (62.0%)	22 (68.8%)	24 (52.2%)	55 (64.7%)	0.219	0.225	0.847
Fatigue (n=158)	102 (62.6%)	18 (56.2%)	30 (65.2%)	54 (63.5%)	0.877	0.939	1.000
DMT*							
Infusions	20 (12.3%)	2 (6.2%)	9 (19.6%)	9 (10.6%)	0.427	0.039	0.067
Injectables	40 (24.5%)	7 (21.9%)	10 (21.7%)	23 (27.1%)			
Never/None/Other	46 (28.2%)	14 (43.8%)	16 (34.8%)	16 (18.8%)			
Oral	57 (35.0%)	9 (28.1%)	11 (23.9%)	37 (43.5%)			
Serum Vitamin D - Initial (ng/dl) (n=158) **	30.1 (16.2)	30.7 (18.9)	30.0 (14.5)	29.9 (16.2)	0.991	0.975	0.841
Serum Vitamin D - Final (ng/dl) (n=146) **	53.8 (24.1)	49.5 (23.4)	47.2 (17.7)	58.3 (26.1)	0.924	0.008	0.106
Serum Calcium - Initial (mg/dl) (n=162) **	9.27 (0.396)	9.25 (0.472)	9.27 (0.378)	9.28 (0.380)	0.826	0.817	0.762
Serum Calcium - Final (mg/dl) (n=148)**	9.32 (0.425)	9.36 (0.411)	9.33 (0.436)	9.29 (0.428)	0.630	0.659	0.443

* Fisher's exact test
** Wilcoxon rank test

Injectables - Interferon-Beta1a (Avonex), beta-interferon (Betaseron), and glatiramer acetate (Copaxone)

Oral - sphingosine 1-phosphate receptor modulator (fingolimod), dimethyl fumarate (Tecfidera), teriflunomide (Aubagio) Infusions - Natalizumab, Rituximab, Ocrelizumab

Table 2. Clinical Outcomes including time-to-change to EDSS score 6 and Deaths of veterans with MS prescribed by groups (Mean (SD) or n (%), where applicable)

Grouping Variables (N)	Total Population (N=144)	Vitamin D supplementation			P-value		
		1000 IU/day (N=26)	2000 IU/day (N=37)	4000 IU/day (N=81)	1000 vs. 2000 IU/day	2000 vs. 4000 IU/day	1000 vs. 4000 IU/day
Initial EDSS score (n=143) [I] *	3.63 (2.85)	3.70 (2.94)	4.54 (2.88)	3.19 (2.75)	0.217	0.019	0.442
Final EDSS score (n=141) [F] *	4.91 (2.79)	5.06 (3.19)	5.16 (2.80)	4.76 (2.68)	0.924	0.477	0.666
Change EDSS (n=140) [F-I] *	1.30 (1.96)	1.30 (1.98)	0.657 (1.10)	1.58 (2.18)	0.337	0.003	0.558
Initial TFIM score (n=137) [I] *	111 (18.1)	108 (19.4)	109 (16.2)	112 (18.5)	0.627	0.301	0.312
Final TFIM score (n=140) [F] *	104 (23.9)	100 (24.8)	105 (19.6)	105 (25.4)	0.540	0.988	0.351
Change TFIM (n=133) [F-I] *	-6.41 (17.5)	-8.42 (20.5)	-5.12 (17.7)	-6.34 (16.5)	0.497	0.736	0.655
Change to EDSS score 6 (n=136)	80 (55.6 %)	17 (65.4 %)	22 (59.5 %)	41 (50.6 %)	1.000	0.158	0.237
Time to Change to EDSS score 6 (n=56) *	7.20 (5.32)	7.00 (5.73)	5.40 (4.70)	7.71 (5.42)	0.558	0.199	0.754

Deaths	21 (14.6%)	6 (23.1%)	9 (24.3%)	6 (7.4%)	1.000	0.024	0.065

MMSE: Mini Mental State Examination
EDSS: Expanded Disability Severity Scale
TFIM: Total Functional Independence Measure
* Wilcoxon rank test

Table 3. Clinical Outcomes including time-to-change to EDSS score 6 and Deaths of veterans with MS prescribed by groups for 5-years or more. (Mean (SD) or n (%), where applicable)

Grouping Variables (N)	Total Population (N=107)	Vitamin D supplementation			P-value		
		1000 IU/day (N=21)	2000 IU/day (N=24)	4000 IU/day (N=62)	1000 vs. 2000 IU/day	2000 vs. 4000 IU/day	1000 vs. 4000 IU/day
Initial EDSS score (n=106) [I] *	3.80 (2.83)	3.75 (2.77)	5.06 (2.79)	3.33 (2.75)	0.071	0.013	0.560
Initial EDSS score (n=107) [F] *	5.30 (2.71)	5.29 (3.00)	5.92 (2.65)	5.06 (2.63)	0.357	0.188	0.766
Change EDSS (n=106) [F-I] *	1.49 (2.05)	1.48 (2.17)	0.854 (1.15)	1.73 (2.24)	0.611	0.019	0.649
Initial TFIM score (n=100) [I] *	111 (17.1)	110 (15.9)	107 (14.6)	113 (18.3)	0.402	0.153	0.502
Final TFIM score (n=107) [F] *	103 (24.1)	102 (21.4)	101 (21.4)	104 (26.1)	0.900	0.600	0.740
Change TFIM (n=100) [F-I] *	-8.27 (19.2)	-9.00 (22.4)	-7.27 (21.0)	-8.41 (17.7)	0.814	0.823	0.917
Change to EDSS score 6 (n=106)	72 (67.3%)	15 (71.4%)	19 (79.2%)	38 (61.3%)	1.000	0.187	0.397
Time to Change to EDSS score 6 (n=34) *	10.0 (4.93)	9.80 (5.50)	8.20 (5.26)	10.5 (4.88)	0.598	0.414	0.813
Deaths	18 (16.8%)	5(23.8 %)	8 (33.3%)	5 (8.1%)	0.709	0.009	0.127

MMSE: Mini Mental State Examination
EDSS: Expanded Disability Severity Scale
TFIM: Total Functional Independence Measure
* Wilcoxon rank test

Table 4. Clinical Outcomes including time-to-change to EDSS score 6 and Deaths of veterans with MS prescribed by groups with serum level of 25[OH]D. (Mean (SD) or n (%), where applicable)

Grouping Variables [N]	Total population (N=59)	Serum level of 25[OH]D		p -value
		<32 ng/ml (N=43)	≥32 ng/ml (N=16)	
Initial EDSS score (n=59) [I]	3.55 (2.88)	3.65 (2.81)	3.28 (3.15)	0.612*
Final EDSS score (n=58) [F]	4.91 (3.00)	4.93 (2.99)	4.88 (3.12)	0.868*
Change EDSS (n=58) [F-I]	1.34 (1.97)	1.24 (2.02)	1.59 (1.86)	0.450*
Initial TFIM score (n=56) [I]	112 (17.5)	112 (16.7)	112 (19.7)	0.573*
Final TFIM score (n=57) [F]	103 (25.8)	103 (27.3)	104 (22.1)	0.972*
Change TFIM (n=54) [F-I]	-8.63 (16.7)	-8.89 (18.3)	-8.00 (12.7)	0.940*
Change to EDSS score 6 (n=53)	30 (50.8%)	21 (48.8%)	9 (56.2%)	1**
Time to Change to EDSS score 6 (n=23)	7.52 (5.46)	7.88 (5.64)	6.71 (5.35)	0.657*
Deaths	10 (16.9%)	9 (20.9%)	1 (6.2%)	0.259**

MMSE: Mini Mental State Examination
EDSS: Expanded Disability Severity Scale
TFIM: Total Functional Independence Measure
* Wilcoxon rank test
** Multiple sclerosis not included in the Fisher's exact test
*** Fisher's exact test

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