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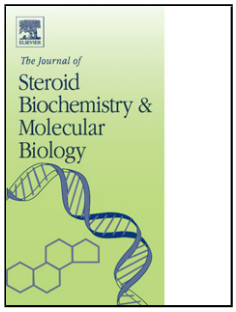

Serum Vitamin D Level is Associated with Speed of Processing in Multiple Sclerosis Patients

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Title Page

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Highlights

- Multiple Sclerosis (MS) is associated with processing speed impairment.
- MS patients with deficient 25(OH)D vs. sufficient have slower processing speed
- 25(OH)D changes in MS could predict changes in processing speed
- Clinical monitoring and supplementation of 25(OH)D in MS is reinforced

Abstract

Multiple Sclerosis (MS) is often associated with low serum 25(OH)D levels, as well as cognitive dysfunctions. The relationship between 25(OH)D and the most commonly affected cognitive domain in MS; processing speed, is poorly explored. The purpose of this study is to: (1) assess the effect of serum 25(OH)D change on processing speed in MS, and (2) explore the relationship

between serum 25(OH)D and brain volume changes in MS. A retrospective chart review was conducted, data from 299 patients were extracted (baseline), of whom 163 had follow-up measurements (after at least a 9-month interval). The Symbol Digits Modalities Test (SDMT) was used as a measure of processing speed. MRI data was available from 78 individuals at baseline, and 70 at follow-up. SDMT scores and brain volumes (Cerebellum (total, grey, and white), intracranial, Grey Matter (GM), and White Matter (WM)) were compared based on 25(OH)D levels and their changes towards follow-up. Results indicated that patients with deficient 25(OH)D levels had lower SDMT scores when compared to those with sufficient levels, and SDMT scores improved as a function of 25(OH)D. For MRI measures, only patients with sufficient 25(OH)D levels during both assessment periods had significant changes in intracranial and total cerebellum volumes. We conclude that 25(OH)D levels seem to have an effect on processing speed in MS, thus the importance of clinical monitoring and supplementation in this regard is reinforced.

Keywords: *Vitamin D; serum 25(OH)D; Processing Speed; SDMT; cognitive functioning; Multiple Sclerosis.*

Background

Multiple Sclerosis (MS) is an autoimmune disorder of the central nervous system. Persons with a genetic predisposition or who are exposed to a combination of environmental risk factors such as lower 25 hydroxyvitamin D [25(OH)D] levels are at a higher risk of developing MS [1]. Low 25(OH)D is highly prevalent in the Middle East. To date, several studies support the association of low serum 25(OH)D and MS [2-5] yet it remains inconclusive. A 2018 Cochrane systematic review, including a small number of randomized controlled trials (RCTs) and quasi-RCTs concluded with little confidence no benefits of vitamin D supplementation on disease activity in MS [6]. A recent report, however; using Mendelian randomization analyses, suggested that low serum 25(OH)D is a cause of MS, independent of other established risk factors [7]. Furthermore, several observational studies of patients with MS found that increased 25(OH)D levels may lead to fewer relapses [8-10], decreased magnetic resonance imaging (MRI) activity [11], decreased regional and global brain atrophy [12, 13], and most importantly better cognitive performance [14].

Vitamin D is recognized as a neuro-steroid [15] in addition to its well-known role in mineral homeostasis and bone function [16, 17]. In the brain, neurons and glial cells express the associated receptor (1,25-dihydroxyvitamin D3 receptor, VDR) [18]. Such an expression suggests a role for vitamin D in neurocognitive function [15-17, 19, 20]. Moreover, cross-sectional and prospective population-based studies, such as the EPIDOS study, found that the odds of global cognitive impairment and risk of global cognitive decline are higher with low serum 25OHD levels [16, 17, 19-26]. Our group has previously reported an association between low 25(OH)D levels and cognitive impairment in adults [27].

Although symptoms of MS can vary significantly from one patient to another [28], cognitive impairment occurs in 40 - 65% of MS patients, and profoundly impact their quality of life and functionality. Cognitive impairment in this population generally affects complex attention, information processing speed, memory, and executive functions [29-31]. Currently, limited data links vitamin D supplementation, consequently higher serum 25(OH)D levels, to improved cognitive function, or slower cognitive decline in MS, and the research results were inconsistent [30]. Our group has previously investigated the effect of vitamin D_3 replacement on cognition in

MS. The participants who received three months vitamin D_3 supplementation showed improvements in most cognitive tests at follow-up

Impaired speed of processing is frequently exhibited in MS patients [32-35]. Among the most commonly used measures of processing speed is the Symbol Digit Modalities Test (SDMT) [34, 36], a sensitive test that is highly correlated with the global cognitive function of MS patients and often used clinically to monitor the patients' cognitive performance over time [32-34].

We have observed very low 25(OH)D levels in several of our MS patients at the MS center as the neurologists systematically measure serum 25(OH)D clinically and institute supplementation until they reach a sufficiently high level of serum 25(OH)D. To date, there is no consensus on the optimal level of serum 25(OH)D level in adults and MS patients in particular [37, 38].
All patients followed at our MS center are tested using the SDMT at every (6 months) visit.

Thus, the primary aim of this study was to assess the effect of change in serum 25(OH)D on the speed of processing of MS patients between two clinical visits of at least nine months apart. We hypothesized that MS patients with higher serum 25(OH)D would demonstrate improved speed of processing.

The secondary aim was to explore the change in total Gray (GM) and White Matter (WM) volumes and cerebellum [39] between the two visits and assess for a possible relationship between serum 25(OH)D, brain volumes and SDMT change.

Methods

Design and Sample

This study is a retrospective chart review within a larger ongoing longitudinal prospective cohort study conducted at the Nehme and Therese Tohme Multiple Sclerosis Center of the American University of Beirut Medical Center (AUBMC). The Institutional Review Board of the American University of Beirut approved this study, and all participants gave written informed consent before participating.

Inclusion and Exclusion

Eligible patients included in this analysis were seen at the MS center between 2012 and 2018, aged 16 years or older, who had SDMT and serum 25(OH)D measurements performed not more than three months apart to control for seasonal-related fluctuation in serum 25(OH)D level and cognitive responses [40, 41], and 25(OH)D levels < 100 ng/mL at the first visit. The subjects also needed to have a second set of SDMT and serum 25(OH)D measurements at least 9 months later, also the two tests should be within 3 months apart. Further inclusion criteria included the following: diagnosis with Radiological Isolated Syndrome (RIS), Clinically Isolated Syndrome (CIS), Relapsing-Remitting MS (RRMS), or Secondary Progressive MS (SPMS) based on the 2005 revised McDonald criteria [42]. There were no exclusion criteria for Disease-Modifying Therapy (DMT). Data from patients were included if they were not on DMT or any DMT. **Figure 1 (part A)** is a flow chart representing the data extraction steps, and the number of cases at each phase, leading to the final sample size.

Data Collection Measures

Sociodemographic (age, sex, education, and marital status), clinical (MS disease type and duration, EDSS, DMT intake (yes/no), height and weight; used to compute Body Mass Index

(BMI), serum 25OHD level (ng/mL), depression (present/not present), smoking activity (smoker/non-smoker) - and type (cigarette or hubble-bubble), brain imaging information (e.g. number of new lesions) [43-50], and SDMT scores were collected from their medical charts and the computerized hospital information system.

SDMT

The SDMT shows robust psychometric properties (ex. test-retest), with low practice effects [51]. Like most tests, performance on the SDMT can be influenced by several factors such as visual acuity and ocular motor functions. In addition, some incidental learning of the key and symbol pairings can occur (alternative versions are present) [36, 51]. In this study, the speed of processing was assessed by the same trained MS nurses (inter-rater reliability of 0.85, routine practice) using the SDMT (oral version, alternate forms every 6 months) [52]. In 90 seconds, participants are asked to replace, as quickly as possible, a set of symbols with numbers based on a key. The key is composed of nine symbols, each associated with a digit (1 to 9) [52].

Serum 25(OH)D measurement

The majority of the serum 25(OH)D levels were measured in the Pathology and Laboratory Medicine Department at AUBMC using the Roche® Diagnostics total assay (baseline n=183 (65.6%) and follow-up n=110 (70.5)). However, due to various financial coverage types, or remoteness from the medical center, some patients could have performed the test in an outside laboratories using either the Roche Diagnostics total assay or the second most commonly used method, the DiaSorin LIAISON® 25OHD total assay (DiaSorin, Stillwater, MN, USA) (baseline n= 96 (34.4%) and follow-up n=46 (15.4))[53]. In this study we considered patients with 25 (OH)D < 25 ng/mL deficient and ≥ 25ng/mL sufficient.

Magnetic Resonance Imaging (MRI)

Acquisition

All patients had their MRIs performed at the AUBMC between June-2013 and December-2018. MRI acquisitions were performed on a 3T Philips Ingenia System (Philips Healthcare, Best, The Netherlands) using a 16-channel head coil. MRI protocol consisted of 2D gradient-echo (GE) axial T1-weighted (TR/TE: 260/204ms; Flip angle 80°; slice thickness 3mm) or 3D sagittal T1-weighted with and without gadolinium injection (0.1mmol/kg of gadoterate-meglumine) (TR/TE: 8.3/3.8ms; Flip-angle 8°; slice thickness: 1mm), depending on the availability. The MRI protocol for MS patients at the centre has been updated to include 3D T1-weighted images instead of 2D images in 2016. Thus, some MRIs were acquired with a 2D GE T1, and the rest were acquired using 3D T1.

Image processing

An experienced operator (author SH) with more than ten years of experience first performed a quality control step on all MRIs to rule out and exclude any images with major artifacts that could introduce errors or biases during image segmentation. Image processing was performed on our institution vLabs Virtual Workstation. A bias field correction on all T1-weighted images using the N4-algorithm in 3DSlicer (https://www.slicer.org/) was first performed, followed by a whole-brain extraction using the Brain Extraction Tool of the FMRIB Software Library (FSL5.0) to measure the intracranial volume (ICV) [54]. Total gray (GM) and white matter (WM) volumes were then estimated using SIENAX [55]. A publicly available MRI brain volumetry system (volbrain: http://volbrain.upv.es) was also used for volumetric assessment of the cerebellum [56]. More in

details, T1-weighted images were fed into CERES, an automatic pipeline for Cerebellum analysis, that delivered volumes of the main cerebellar tissues (WM and GM). The number of new T1 enhancing lesions (at Baseline and Follow-up), and T2 lesions (at Baseline), were extracted from medical records.

Statistical analysis

Descriptive statistics were computed and presented as means (M) and standard deviations (SD) for all continuous variables, and frequencies (F), and percentages (%) for all categorical variables. Independent t-test was used to compare SDMT scores between the two groups of 25 (OH)D deficient vs. sufficient patients on the first visit. To control for the effect of DMTs on change in SDMT score the same comparison was performed between the two groups including only the individuals who were receiving any DMT during their first visit to the centre or at follow-up. Paired t-tests were then performed to compare the SDMT scores within patients at the two time-points.

Three stepwise linear regression models were used: 1) To identify predictors of the SDMT scores at Baseline, whereby the independent variable 25(OH)D category (Baseline) (sufficient vs. deficient) was included with the following possible confounders: age, education (< 12 years vs. ≥ 12 years), EDSS (Baseline), DMT intake (yes vs. no) (Baseline), MS type (Baseline), and disease duration (in months; Baseline); 2) the same analysis was repeated with 25(OH)D entered as a continuous variable; **3**) To identify the independent predictors of $SDMT_{diff}$ (SDMT Follow-up score – SDMT Baseline score), the independent variable difference in 25 (OH)D level (Follow-up – Baseline) was included with the following potential confounders: EDSS (Follow-up), MS diagnosis (Follow-up), age (Follow-up), education (< 12 years vs. ≥ 12 years), DMT intake (Follow-up) (yes vs. no), disease duration (in months; Follow-up). All statistical assumptions were checked as appropriate for both models (ex. Independence of residuals using Durbin-Watson, and multicollinearity using Tolerance and Variance Inflation Factor). Both models did not include BMI and smoking as potential independent variables; which did not differ between 25(OH)D deficient and sufficient groups (BMI: $t(259) = 1.468$, $p = 0.143$; smoking: $t(293) = 0.161$, $p = 0.872$). The same analysis was repeated using the same models showed that BMI and smoking do not predict any of the outcome variables of interest; these values were excluded by both stepwise linear regression analyses with p of B values > 0.05.

To compare the patients' SDMT scores (at Baseline and Follow-up) based on their respective 25(OH)D status, we performed two sets of Repeated Measures Analysis of Covariance (RM-ANCOVA). Time in months (Baseline (T1) and Follow-up (T2)) served as within-subject variable and Group (MS patients with deficient/sufficient 25(OH)D condition) as between-subject variable. We used age as a co-variable since it was the only significant predictor in the linear regression described earlier. The first model included individuals whose 25(OH)D levels were deficient at baseline, but sufficient at follow-up. The second model included individuals whose 25(OH)D levels were sufficient at both time points (11 individuals were excluded from the analyses as their levels remained deficient or changed from sufficient to deficient at follow-up). The analytical models controlled for participants' age, as well as the time (months) between both measurements. Both of these variables were centered around their means [57-60]. To control for the effect of DMT on SDMT performance the same analysis was conducted on patients who received DMT at follow-up (disregarding baseline DMT), while the second included individuals who received DMTs at both time points. Also, to partial-out the effects of the covariates (age and time between tests) on the SDMT performance, each set of RM-ANCOVA included two analyses. The first RM-

ANCOVA included all covariates, while the second did not. As such, the homogeneity of the regression slopes assumption was checked [61-63].

The Mann-Whitney U-test was used to compare the normalized volume values of the cerebellum (total, grey, and white matter), intracranial, GM, and WM, between the 25(OH)D deficient and sufficient groups. A chi-square test was performed to examine the differences in the number of enhancing T1 lesions at Baseline between the 25(OH)D groups (deficient vs. sufficient).

The Wilcoxon signed-rank tests were used to analyse the MRI data since the data violated the assumption of normality. Raw values of the Cerebellum (total, GM, and WM), intracranial, GM, and WM were compared between Baseline and Follow-up. The same tests were performed for individuals who at both times had sufficient 25(OH)D levels, and for individuals who had deficient levels at Baseline, but sufficient levels at Follow-up. Bonferroni adjustments were applied to correct for multiple comparisons **in this** test, and a p-value of < 0.008 was considered statistically significant.

Stepwise linear regression was used to identify predictors of the MRI brain region volumes with independent variable 25(OH)D category (deficient vs. sufficient) and potential confounding variables: age, BMI, and DMT intake at T1 (yes vs. no). All assumptions were checked.

Statistical analysis was performed using SPSS version 23. Statistical significance is reported as $p<0.05$ except for the Wilcoxon signed-rank tests, which compared MRI measures between baseline and follow-up, whereby the p-value was set at 0.008.

Results

A summary of the analyses performed, and results can be found in Table 5 (supplementary).

Sample characteristics and available data across Baseline and Follow-up

Of the 299 subjects included in this study at Baseline, 163 (54.5%) subjects had available SDMT and 25(OH)D measurements - after 18.26 ± 8.56 months (**figure 1 part A**, and **figure 2**). MRI data was available for 78 subjects at baseline (26.09% of Baseline sample) and 70 subjects at follow-up (**figure 1 part B**). Sociodemographic, cognitive, and clinical information, including MRI, of the study cohort, can be found in **table 1**. 93% of individuals who were deficient with 25(OH)D at baseline, were supplemented with the vitamin towards the follow-up.

In this study, we did not exclude the data of patients who were experiencing a relapse within one month of any visit. However, we randomly selected 60 patients (20%) of the total sample to explore the data of those who experienced an MS relapse. 10% of the 60 (i.e., n = 6) had a relapse around the baseline measurement, 32 of the 60 had a follow-up, 6.25 % of them were experiencing a relapse (i.e., 2 from 32). A Mann-Whitney *U* test to compare the mean rank SDMT scores of individuals with relapse at baseline with their counterparts who were stable at the same timepoint (n = 54) did not show a significant difference between the two groups ($U = 114$, $p = 0.237$).

Change in 25(OH)D, SDMT, and MRI measures from baseline to follow-up

Figure 1, part C shows the change in 25(OH)D status from baseline to follow-up. Overall, 25(OH)D levels improved by 12.3 ng/mL between the two time points ($t(162) = 6.156$, $p < 0.001$).

SDMT scores improved by 3.36 ± 7.08 ($t(162) = -6.05$, $p < 0.001$) from baseline to follow-up (N = 163). For individuals who were on DMTs during both visits (n = 115), the SDMT scores improved by 2.43 ± 6.91 ($t(114) = 3.76$, $p < 0.001$). For subjects who started to take a DMT after their first visit (n = 37), their SDMT scores improved by 7.24 ± 6.28 ($t(36) = 7.01$, $p < 0.001$).

We found statistically significant changes in brain volumes from baseline to follow-up. The results are summarized in **Table 2 (part A and B)**.

SDMT in relation to 25(OH)D levels

Differences in Baseline SDMT scores based on 25(OH)D groups

At baseline, the MS patients with deficient 25(OH)D scored lower on the SDMT (48.77 ± 13.74) than subjects with sufficient levels (52.81 ± 14.75) ($t(297) = -2.37$, $p = 0.018$), with a M_{diff} of -4.05 ($SE_{diff} = 1.71$, 95% CI -7.40, -0.69). Similar results were obtained when restricting the sample to subjects who were started on a DMT ($t(195) = -2.50$, $p = 0.013$), the M_{diff} was -5.53 ($SE_{diff} = 2.21$, 95% CI -9.89, -1.16), the SDMT score was 48.16 ± 13.65 for the deficient group and 53.69 ± 15.32 for the sufficient group.

Predictors of Baseline SDMT, and $SDMT_{diff}$

At Baseline, several clinical and demographic variables significantly predicted SDMT scores ($F(6, 258) = 24.547$, $p < 0.001$). These variables were the following: age, education, EDSS, MS type, DMT intake, and 25(OH)D category. **Table 3** summarizes the coefficients associated with these variables. For 25(OH)D specifically, sufficient levels were associated with better scores ($B = 3.19$, $p = 0.038$). The final model, including all the significant predictors, explained 34.9% of the variance in SDMT scores (value from adjusted R^2). When the 25(OH)D was entered as a continuous variable, it did not predict the SDMT score. The model was rather significant ($F(5, 259) = 28.223$, $p < 0.001$) due to the following predictors: EDSS, age, education, DMT intake, and MS disease type.

But, as expected, the difference in SDMT scores between Baseline and Follow-up (i.e. $SDMT_{diff}$) was predicted by the change in 25(OH)D level (Follow-up – Baseline) and age and. The regression model in which these variables were retained explained 15.6% of the variance in SDMT scores ($F(2, 138) = 12.79$, $p < 0.001$) (**Table 3**).

Given the known discrepancy between the results of the two vitamin D assays used in this study [53], further analyses were conducted as follows. We found the 25(OH)D levels (deficient vs. sufficient) to be equally distributed across the centers (AUBMC laboratory vs. outside laboratories) ($X2 (1, 279) = 1.562$, $p = 0.211$) (Figure XXX (edit figure numbering after HD revision) depicts 25(OH)D values based on the center). The median value from our center was 31.8 ng/mL and from outside laboratories 26.25 ng/mL. We reanalyzed the data twice. We conducted a subgroup analysis using only the 25(OH)D values collected at our institution laboratory. We found the same results of associations and predicted changes.

We furthermore applied an adjustment by adding 2 ng/mL (average absolute bias between the two assays reported in a previous study [53]), to the sample collected from the outside laboratories and we found the same results as well (Table 5 supplement).

Within-subject differences (and supplementary analyses) of SDMT scores based on 25(OH)D category change

For the RM-ANCOVA, we considered the following two 25(OH)D conditions: deficient at baseline, and sufficient at follow-up (DS) (n = 42), versus sufficient at both timepoints (SS) (n = 108). **Figure 1 part C** includes a description of the 25(OH)D categories at every timepoint, as well as their respective DMT intake.

We did not find a statistically significant interactions between SDMT and 25(OH)D after controlling for covariates (age and time) for individuals who received DMT at follow-up only ($F(1, 31) = 1.908$, $p = 0.177$), or for those who received DMT at both timepoints ($F(1, 103) = 0.138$, $p = 0.711$). The latter model violated the assumption of the homogeneity of regression slopes. However age ($F(1, 103) = 16.38$, $p < 0.001$) showed a significant effect, with an effect size of 0.14 (partial Eta^2) possibly contributing to this violation. When the covariates (age and time) were removed from this model, we still did not find a significant interaction between SDMT and 25(OH)D ($F(1, 105) = 0.247$, $p = 0.620$).

To better understand the relationship between SDMT scores and 25(OH)D conditions, and given the small sample size per group, we resorted to non-parametric methods. The results of the Wilcoxon signed-rank test showed that SDMT scores significantly increased in both conditions (**Table 4**). **Figure 3** represents SDMT scores at Baseline and Follow-up for each condition using boxplots. **Figure 4** depicts the change in SDMT scores based on the baseline 25(OH)D category. Both figures include data from two groups (25(OH)D sufficient at both time points, and deficient at baseline but sufficient at follow-up).

MRI measures in relation to 25(OH)D levels

Differences in baseline brain volumes and T1 enhancing lesions based on 25(OH)D groups

There were no significant differences in brain volumes at baseline between 25(OH)D deficient and sufficient groups ($p > 0.05$). Similar results were obtained when the number of T1 enhancing lesions at baseline were compared ($X^2(3) = 4.607$, $p = 0.203$).

Predictors of baseline brain volumes

One variable was retained within each of the following step-wise regression models: intracranial volume ($F(1,65) = 12.003$, $R^2 = 0.156$, $p = 0.001$), cerebellum WM volume ($F(1,65) = 6.710$, $R^2 = 0.094$, $p = 0.012$), and total GM volume ($F(1,65) = 7.176$, $R^2 = 0.099$, $p = 0.009$). The concerned predictors for the three brain volumes were BMI or age. For intracranial volume, B value of BMI was 13680.86 (Standard Error of the Estimate = 144020.46, $p = 0.001$). For cerebellar WM volume, B value of age was -0.032, $p = 0.012$. For total GM volume, B value of age was -0.135 ($p = 0.009$).

Within-subject differences of brain volumes based on 25(OH)D category change

We conducted a subgroup analysis and found that patients who had sufficient 25(OH)D levels at both times (n = 49) were similar to the total sample with significant changes in intracranial and total cerebellum volumes present (based on $p < 0.008$; **table 2**). However, for patients whose 25(OH)D levels changed from deficient at baseline, to sufficient at follow-up, brain volume measures did not differ between the two-time points (n = 11, all $p > 0.008$).

No significant association was found between MRI measures and SDMT scores at both time points.

Discussion

The main findings of this study are that MS subjects deficient in serum 25(OH)D have reduced processing speed compared to patients with sufficient levels. Furthermore, vitamin D_3 supplementation contributes to a significant improvement in processing speed [36]. The significant improvement in SDMT performance was noted in all groups but was more prominent in subjects who started with deficient 25(OH)D levels and became sufficient at follow-up.

When examining the effect of changes in both serum 25(OH)D levels and SDMT scores after controlling for confounding variables, we found that a positive difference in 25(OH)D levels between the two visits was associated with a significant improvement in SDMT performance. We found similar results in patients receiving DMTs. These results indicate an association between serum 25(OH)D levels and speed of processing but cannot be compared with other cognitive domains (refer to Table 5 of Darwish et. al (2017). Despite being a distinct cognitive function, studies showed that information processing is strongly associated with other cognitive domains and could influence other cognitive performance [64, 65].

It is difficult to predict from our results if the serum 25(OH)D levels and its' association with the speed of processing performance reaches a plateau over time. The cut-off serum 25(OH)D level that predicts deterioration in the speed of processing or other cognitive functions is also worth investigating. Future well-designed longitudinal studies with repeated measures including speed of processing and other measures of cognitive domains may answer these questions.

Additionally, it can be argued that the improvement in SDMT scores was due to practice or learning effects. While such effects cannot be entirely ruled out, it remains unlikely given the use of alternative forms in our sample, and the interval of minimum 9 months between both assessments [51, 66, 67].

A score of 55 or below on the SDMT accurately categorizes the patients as cognitively impaired [68], our subjects with deficient 25(OH)D had a median score of 50 that improved to 53 after vitamin D replacement within a relatively short interval of time. We did not collect data as to whether this improvement was noticeable or impacted the patients' daily functions and quality of life, but future studies should include these data.

Previous studies reported that greater disability scores on the EDSS, increased age, and progressive MS type were associated with worst speed of processing performance [69-71], and higher education was associated with better SDMT performance [72]. In our data, only age and change in 25(OH)D levels were significant predictors of the change in SDMT performance. This change in SDMT performance was seen within a year in MS patients while age remains a significant predictor of this change. However, the time interval is short enough not to see a significant influence of other factors such as EDSS and MS type [66, 73]. Also, the effect of education (more or less constant variable) seen at baseline may not have an effect on the change in performance as much as other factors within a one-year period.

We found that age, age interaction with time, and education are consistently significant predictors of speed of processing as previously reported [34, 72], but, we could not explore the

effect of time and 25(OH)D levels on processing speed due to the small sample sizes in each condition. However, the nonparametric analysis we have conducted is promising and showed that indeed a change in 25(OH)D level is associated with higher SDMT scores.

We also found that BMI and not vitamin D was associated with brain volume changes in MS, in line with the findings by Mowry et al. (2018), but we found no association of BMI with processing speed.

The sample used to explore the differences in MRI parameters between the two groups was small, and the time interval between the readings is short. Nonetheless, we found that certain brain regions showed significant and positive change between baseline and follow-up, while others showed the opposite [74]. These changes are worth exploring in a larger sample over a more extended period in a more homogeneous sample of MS patients.

Our study has limitations. First, we did not conduct a sample size calculation a priori but rather conducted a posthoc power analysis using our findings on SDMT performance differences in MS patients (M_{diff} of 3.36, and SD of 7.08) that showed a power of > 0.90. Second, the group of MS patients was heterogeneous, encompassing patients with relapsing-remitting and progressive disease course and on various types of DMTs. Therefore, we could not explore whether the effect of vitamin D_3 supplementation is enhanced under one DMT compared to another. Furthermore, the amount of vitamin D_3 supplementation is variable, and we did not account for other sources of vitamin D such as food or sun exposure. Also, in future studies, it would be better to use the same vitamin D assay in the analysis.

A randomized clinical trial to compare the effect of low versus high dose vitamin D on cognitive performance is ongoing at our center to address some of these questions. Future studies to identify the optimal serum level of 25(OH)D necessary for neuroprotective effects are warranted.

In conclusion, these data emphasize the neuro-steroid function of vitamin D and its' effect on cognitive function in MS patients. It also reinforces the importance of clinical monitoring of 25(OH)D levels and treatment. Vitamin D_3 replacement early in the disease could make a significant change in cognitive function, as suggested by the results of this study.

Author statement

Hala Darwish: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Project administration, Supervision. **Natali Farran:** Software, formal analysis, Writing - Original Draft, Writing - Review & Editing. **Salem Hannoun:** Software, Resources. **Natalie Tadros:** Software, formal analysis. **Bassem Yamout:** Writing - Review & Editing, Resources. **Nabil K. El Ayoubi:** Writing - Review & Editing, Resources. **Samia J. Khoury:** Writing - Review & Editing.

Disclosure/conflict of interest

None.

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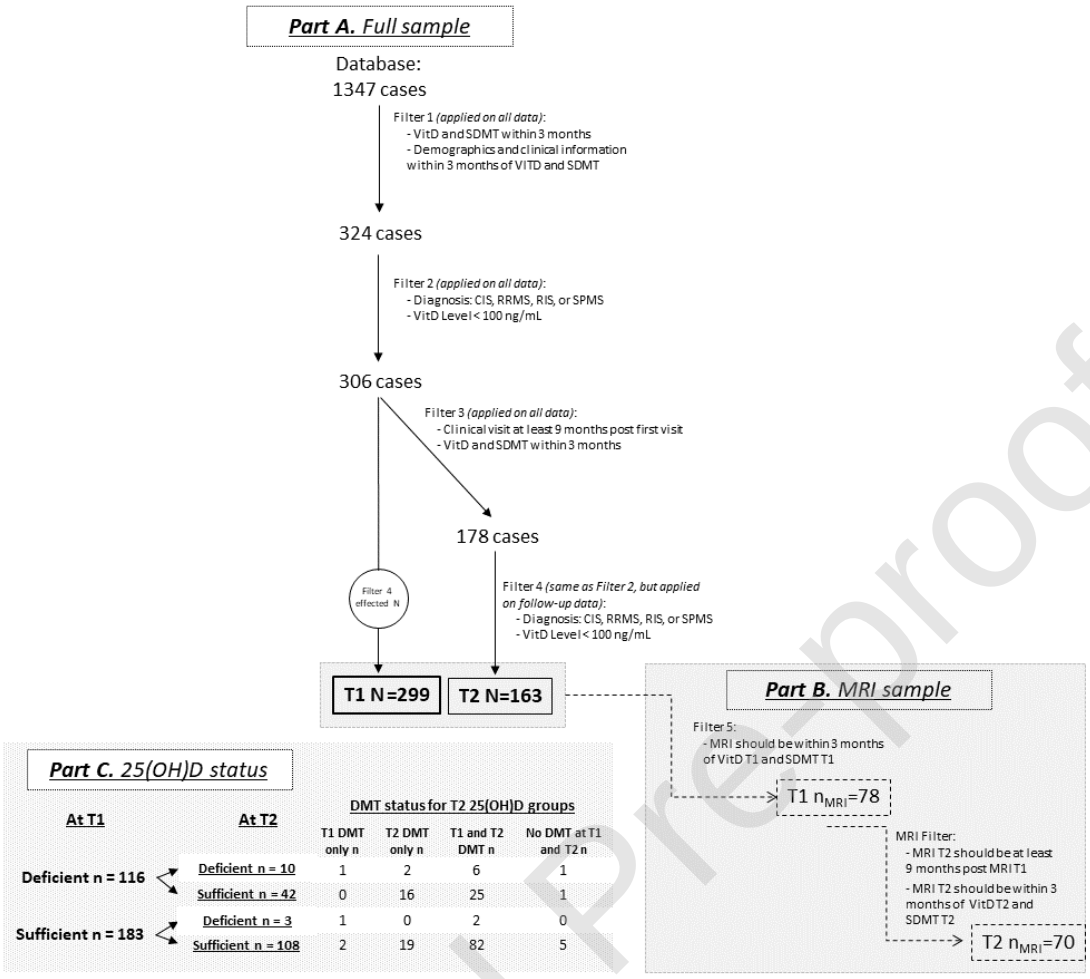
Figure 1. Data extraction flow chart and 25(OH)D status details.



T1: Baseline measurements.
T2: Follow-up measurements.

Figure 2. Time between baseline and follow-up for each patient (n = 163).

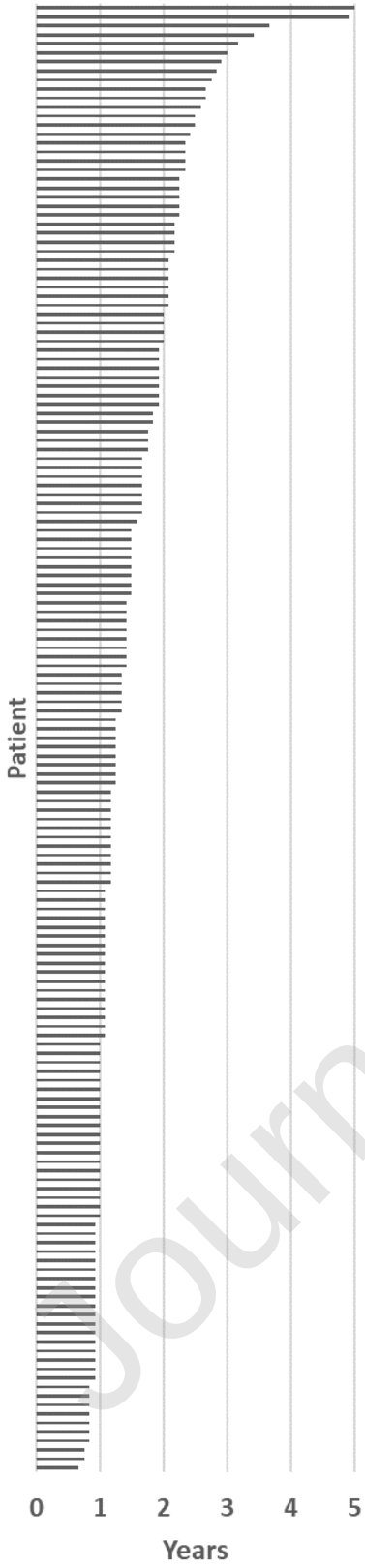
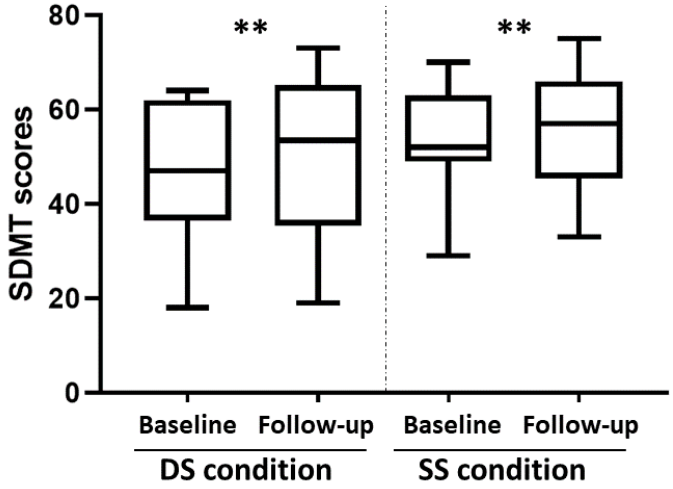
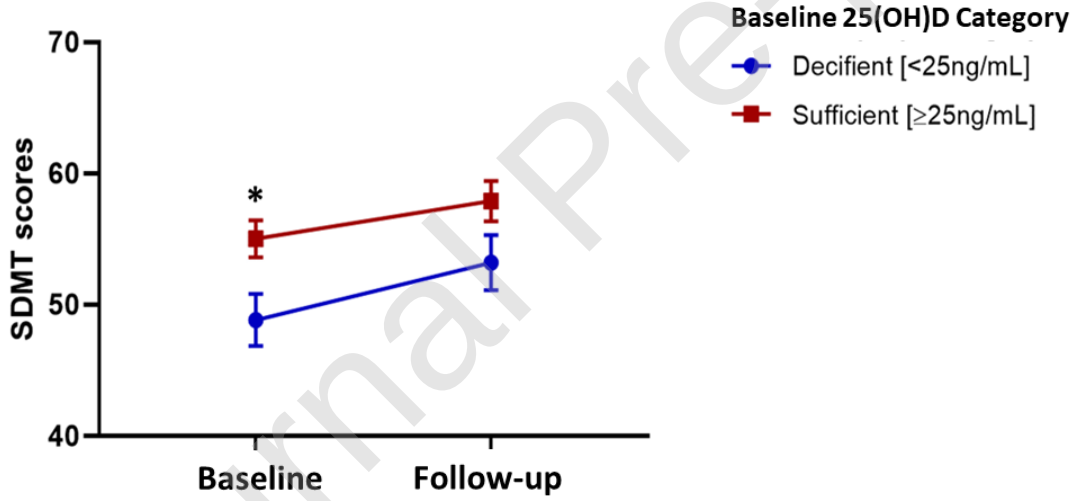


Figure 3. Boxplots for SDMT scores at Baseline and Follow-up for two 25(OH)D conditions.



DS: Deficient 25(OH)D levels at Baseline, and sufficient levels at Follow-up
SS: Sufficient 25(OH)D levels at Baseline, and sufficient levels at Follow-up
***Significant differences in SDMT between visits with p < 0.01*

Figure 4. Mean SDMT scores at Baseline and Follow-up.



Lines represent change in SDMT scores from Baseline to Follow-up, based on Baseline 25(OH)D category
Error bars represent SEM
**Significant differences in SDMT on Baseline between groups with p < 0.05*

Table 1. Sociodemographic, cognitive, and clinical information

	Baseline		**Follow-up**	
	M	***SD***	***M***	***SD***
Age	34.23	10.80	35.50	10.02
	F	***%***	***F***	***%***

Sex				
Female	196	65.55	103	63.20
Male	103	34.45	60	36.80
Education				
< 12 years	10	3.76	1	0.7
≥ 12 years	256	96.24	141	99.30
Marital Status				
Single	111	37.63		
Engaged/Married	172	58.31		
Divorced/Widowed	12	4.06		
	F	***%***	***F***	***%***
MS Disease Type				
CIS	21	7.02	6	3.68
RRMS	262	87.63	148	90.8
RIS	4	1.34	2	1.23
SPMS	12	4.01	7	4.29
	M	***SD***	***M***	***SD***
Disease Duration [DD]				
DD [months]	77.18	82.12	79.09	76.26
DD [years]	6.43	6.84	6.17	6.34
SDMT	51.24	14.48	56.41	16
EDSS	1.61	1.51	1.54	1.62
DMT Intake	***F***	***%***	***F***	***%***
Yes	197	65.89	152	93.3
No	102	34.11	11	6.7
	M	***SD***	***M***	***SD***
BMI	25.27	4.43	25.22	4.69
Vitamin D				
ng/mL*	36.51	22.17	52.37	18.86
Categorized	***F***	***%***	***F***	***%***
Deficient [< 25 ng/mL]	116	38.80	13	7.98
Sufficient [≥ 25 ng/mL]	183	61.20	150	92.02
Depression				
Present	60	20.48	41	25.31
Not Present	233	79.52	121	74.69
Smoking				
Smoker	107	36.27	70	43.21
Non-smoker	188	63.73	92	56.79
Type				
Cigarettes				
Yes	69	23.39	39	24.07
No	226	76.61	123	75.93
Hubble Bubble				
Yes	40	13.56	33	20.37
No	255	86.44	129	79.63

	M	***SD***	***M***	***SD***
MRI [normalized values]**				
Cerebellum [total]	10.23	1.21	9.90	1.08
Gray Matter	2.63	0.37	2.43	0.40
White Matter	7.61	1.03	7.47	0.88
Intracranial	1257689.17	151476.92	1280863.89	151318.16
Gray Matter	41.94	4.40	41.99	3.30
White Matter	41.54	16.27	37.16	3.63
	F	***%***	***F***	***%***
Number of New Lesions				
T1 Enhancing Type				
No Lesions	58	81.69	49	94.2
1 Lesion	7	9.86	2	3.8
2 Lesions	2	2.82	0	0
3 or More Lesions	4	5.63	1	1.9
T2 Type				
No Lesions	44	63.77	NA	NA
1 Lesion	11	15.94	NA	NA
2 Lesions	6	8.7	NA	NA
3 or More Lesions	8	11.59	NA	NA

*There was a statistically significant increase of 12.3 ng/mL in Vitamin D levels towards Follow-up ($t(162) = 6.156$, 95% CI 16.24 to 8.35, $p < 0.001$).
**Normalized values are the percentage of each brain region whereby: (structure*100)/Intracranial Volume.
Abbreviations:
M: Mean.
SD: Standard Deviation.
F: Frequency.
ng/mL: nanograms per milliliter.

Table 2. Differences in MRI volumes* between Baseline and Follow-up for the full sample and for one 25(OH)D condition

MRI measure	**Median**		**Direction of change**	**Z**	**p****
	Baseline	**Follow-up**			
Part A. For all patients (raw values***)					
Cerebellum [total][a]	126080.86	123304.89	Decrease	-2.730	**0.006**
Gray Matter[a]				-2.505	0.012
White Matter[b]				-0.066	0.508
Intracranial[b]	1247937	1260734.13	Increase	-2.780	**0.005**
Gray Matter[b]				-2.061	0.039
White Matter[a]				-2.405	0.016
Part B. For patients with sufficient 25(OH)D at Baseline and Follow-up (raw values***)					
Cerebellum [total][a]	124727.18	123265.84	Decrease	-2.840	**0.005**
Gray Matter[a]				-1.766	0.077

White Matter[b]				-0.244	0.807
Intracranial[b]	1231516.88	1257888	Increase	-2.581	0.01
Gray Matter[b]				-1.756	0.079
White Matter[a]	481706.57	449435.17	Decrease	-2.999	**0.003**

**Results displayed are for Wilcoxon signed-rank tests (parts A and B). Medians are present for significant results*
***Bonferroni adjustments were applied to correct for multiple comparisons, and a p value of < 0.008 wa considered as statistically significant.*
****Raw values are volumes in mm^3.*
[a]Based on positive ranks.
[b]Based on negative ranks.

Table 3. Regression models for SDMT at Baseline and $SDMT_{diff}$

Predictors of SDMT at Baseline					
Model 1: 25(OH)D as a categorical variable **Adjusted R^2 = 0.349, F(6, 258) = 24.547, p < 0.001** **Variables entered in the model:** 25(OH)D category (at Baseline) (sufficient vs. deficient), age, education (< 12 years vs. ≥ 12 years), EDSS (at Baseline), DMT intake (yes vs. no) (at Baseline), MS type (at Baseline), and disease duration (in months) (at Baseline)					
Coefficients					
Variable	**B**	**Std. Error**	**95% LB**	**95% UB**	**p**
EDSS at Baseline	-3.36	0.49	-4.33	-2.4	<0.001
Age at Baseline	-0.46	0.07	-0.6	-0.32	<0.001
Education*	10.42	3.86	2.83	18.02	0.007
DMT Intake**	3.43	1.53	0.4	6.45	0.026
MS type at Baseline***	-4.06	1.51	-7.03	-1.09	0.008
$25(OH)D_{category}$****	3.19	1.53	0.18	6.2	0.038
Model 2: 25(OH)D as a continuous variable **Adjusted R^2 = 0.34, F(5, 259) = 28.223, p < 0.001**					
Variables entered in the model: 25(OH)D (at Baseline; ng/mL), age, education (< 12 years vs. ≥ 12 years), EDSS (at Baseline), DMT intake (yes vs. no) (at Baseline), MS type (at Baseline), and disease duration (in months) (at Baseline)					
Coefficients					
Variable	**B**	**Std. Error**	**95% LB**	**95% UB**	**p**
EDSS at Baseline	-3.54	.49	-4.50	-2.58	<0.001
Age at Baseline	-0.46	.07	-0.60	-0.31	<0.001
Education*	9.73	3.87	2.11	17.34	0.012
DMT Intake**	3.80	1.53	0.78	6.82	0.014
MS type at Baseline***	-3.64	1.50	-6.60	-0.68	0.016
Predictors of $SDMT_{diff}$					
R^2 = 0.156, F(2, 138) = 12.79, p < 0.001					

Variables entered in the model: 25(OH)D (Follow-up – Baseline; ng/mL), age, education (< 12 years vs. ≥ 12 years), EDSS (at T2), DMT intake (yes vs. no) (at T2), MS type (at T2), and disease duration (in months) (at T2)					
Coefficients					
Variable	**B**	**Std. Error**	**95% LB**	**95% UB**	**p**
Age at Follow-up	-0.21	0.06	-0.32	-0.1	<0.001
$25(OH)D_{diff}$ □□	0.08	0.02	0.04	0.12	0.001
Variable labels used: **Education; 0: < 12 years, 1: ≥ 12 years.*					
***DMT; 0: no intake, 1: yes intake.*					
****MS type; 0: CIS, 1: RRMS, 2: RIS, 3: SPMS.*					
*****$25(OH)D_{category}$; 0: deficient, 1: sufficient.*					
□ *$SDMT_{diff}$ (SDMT Follow-up score – SDMT Baseline score).* □□ *$25(OH)D_{diff}$: 25(OH)D Follow-up value* – 25(OH)D *Baseline value.* *Abbreviations:* *B: Unstandardized Coefficient.* *LB: Lower Bound of Confidence Interval.* *UB: Upper Bound of Confidence Interval.*					

Table 4. Differences in SDMT scores* for two 25(OH)D conditions

25(OH)D condition	**Median**		**Direction of change**	**Z**	**p**
	Baseline	**Follow-up**			
Condition 1**: Deficient at Baseline, sufficient at Follow-up	50 (range = 59)	53 (range = 65)	Increase	-4.206	**<0.001**
Condition 2***: Sufficient at Baseline and Follow-up	55 (range = 73)	59 (range = 98)	Increase	-4.674	**<0.001**
**Results displayed are for the Wilcoxon signed-rank tests, and are based on negative ranks. Medians are present for significant results.* ***Median 25(OH)D levels (ng/ml): 15.85 at Baseline (range = 21.5), and 50.2 at Follow-up (range = 73).* ****Median 25(OH)D levels: 50.5 at Baseline (range = 74.4), and 55.3 at Follow-up (range = 72.3).*					