ORIGINAL ARTICLE

Vitamin D is associated with degree of disability in patients with fully ambulatory relapsing–remitting multiple sclerosis

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Background and purpose: Vitamin D deficiency is a recognized risk factor for multiple sclerosis (MS) and is associated with increased disease activity. It has also been proposed that the lower the vitamin D levels are, the higher is the handicap.

Methods: To refine the links between vitamin D insufficiency and disability in MS patients, a retrospective cohort analysis was performed including 181 patients prospectively followed without previous vitamin D supplementation, and age, gender, age at MS onset, MS type, MS activity, Expanded Disability Status Scale (EDSS) were analysed in correlation with plasma vitamin D levels.

Results: Vitamin D levels were significantly higher in relapsing–remitting MS than in progressive forms of MS in multivariate analyses adjusted for age, ethnicity, gender, disease duration and season ($P = 0.0487$). Overall, there was a negative correlation between vitamin D level and EDSS score ($P = 0.0001$, $r = -0.33$). In relapsing–remitting MS, vitamin D levels were only correlated with disability scores for EDSS < 4 ($P = 0.0012$). Patients with >20 ng/ml of vitamin D were 2.78 times more likely to have an EDSS < 4 ($P = 0.0011$, 95% confidence interval 1.49–5.00).

Conclusion: Data support previous work suggesting that vitamin D deficiency is associated with higher risk of disability in MS. Vitamin D levels also correlated with the degree of disability in fully ambulatory patients with relapsing–remitting MS. These additional results support the pertinence of randomized controlled trials analysing the interest of an early vitamin D supplementation in MS patients to influence evolution of disability.

Introduction

Several genetic and environmental factors are known to influence the risk of developing multiple sclerosis (MS). Amongst them, vitamin D deficiency represents one of the most complex to understand [1]. Vitamin D is a pleiotropic hormone that can influence the expression of >200 genes through its cytoplasmic vitamin D receptor (VDR) gene [2]. Most genes regulated by vitamin D are involved in immunity, such as the HLA-DRB1*15 allele, another important risk factor for MS with a highly conserved VDR element in its promoter [3]. Vitamin D deficiency also has synergistic interactions with other risk factors, such as smoking and Epstein–Barr virus infection, and represents a promising approach for primary prevention in children and young adults [4].

However, it is less clear whether vitamin D has a role in the progression of MS. Studies on animal models provide evidence that vitamin D can influence the course of experimental autoimmune encephalomyelitis (EAE) [5]. In progressive models of EAE, 1,25

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$(OH)_2D_3$, the active hormone, can slow the progression of disability [6] and is correlated with a reduced lesion load [7]. Vitamin D has been shown to influence disease activity (as measured by brain imaging) in patients with MS and relapse risk [8]. Finally, a more pronounced vitamin D insufficiency has been reported in relapsing–remitting MS (RRMS) patients with higher levels of disability assessed by the Expanded Disability Status Scale (EDSS) and in patients with progressive forms of MS compared with RRMS, making vitamin D a potential prognostic factor in MS [9,10]. However, this was not confirmed in other studies including fewer patients [11,12].

Vitamin D deficiency has also been described as a potential prognostic factor for other neurological diseases. Patients with low vitamin D levels are at higher risk of developing cognitive impairment, of worsening of Parkinson's disease and of stroke [13,14]. In amyotrophic lateral sclerosis, patients with vitamin D deficiency deteriorated four times faster than other patients [15].

The mechanisms of increased neuronal vulnerability are not completely understood and do not seem to be disease specific. However, demonstrating that vitamin D levels can act as a prognostic factor for MS could have both preventive and therapeutic implications. Herein, this important question is addressed in a series of 181 MS patients.

Materials and methods

The patients' files from our MS clinic were reviewed and the following data were entered into this study when all the criteria were present: date of birth, gender, age at MS onset, MS type [primary progressive MS (PPMS), secondary progressive MS (SPMS) and RRMS], measurement of plasma vitamin D levels (25-hydroxyvitamin D), date of the measurement and EDSS at that time (or <3 months before or after this measurement), geographical origin (European, North African), familial antecedents of MS (defined by the presence of one case in first or second degree relatives) and absence of vitamin D supplementation before measurement. For patients with RRMS, additional criteria were needed: number of relapses since onset and delay between the first two relapses. To determine the type of MS, each patient's file was reviewed by two neurologists and information was included if they both agreed upon the 2005 MS diagnostic criteria [16]. For each patient, the MS severity score (MSSS) score was obtained according to that determined by Roxburgh *et al*. [17]. This study has been approved by the CPP Sud-Méditerranée IV (Institutional Review Board) under number Q-2014-06-01.

The population's characteristics were compared to determine the correlation between vitamin D level and MS phenotype. Statistical analyses first checked whether the data for vitamin D level were normally distributed using the Shapiro–Wilk test. If they were not ($P < 0.0001$), then further statistical analyses were performed using a non-parametric test. The Kruskal–Wallis test compared vitamin D levels amongst MS types and the frequency of relapses. Wilcoxon's test compared vitamin D levels of patients with European and North African ancestry, and the Kruskal–Wallis test was used between groups. A Bonferroni correction was used for multiple testing.

To rule out confounders in group comparisons, bivariate and multivariate analyses were performed. Correlations between EDSS or MSSS and vitamin D levels were studied using Kendall's correlation coefficient. Statistical analyses were performed by MO and JPD using R [18] and SAS statistical software (version 9.3; SAS Institute Inc., Cary, NC, USA).

Results

A total of 181 patients with MS fulfilled all the inclusion criteria. There were 131 women and 50 men, mean age 45.3 years (Table 1). The mean level of vitamin D for the whole population was 20 ng/ml ± 11.9 (SD), which was below the recommended value of 30 ng/ml [1]. Analyses did not reveal any significant differences regarding vitamin D levels between men and women or between cases of sporadic and familial MS. However, patients of North African ancestry did have significantly lower vitamin D levels compared with those of European ancestry (9.95 ± 9.85 vs. 20.5 ± 11.76 ng/ml, respectively, $P = 0.0065$).

Relapsing–remitting MS patients ($n = 122$) had a lower age and EDSS scores, and shorter disease

Table 1 Characteristics of our population with multiple sclerosis (MS)

		Vitamin D levels[a] (ng/ml)	*P* value
N	181	20.0 ± 11.9	
Age[a] (years)	45.3 ± 13.1		
Disease duration[a] (years)	12.1 ± 9.2		
EDSS[a]	3.52 ± 2.38		
Women	131 (72%)	19.5 ± 11.2	NS
Men	50 (28%)	21.3 ± 13.6	
European origin	171 (94.5%)	20.5 ± 11.76	0.0065
North African origin	10 (5.5%)	9.95 ± 9.85	
Non-familial MS	156 (86%)	19.3 ± 11.66	NS
Familial MS	25 (14%)	20.0 ± 11.96	

EDSS, Expanded Disability Status Scale; NS, not significant. [a]Values are mean ± SD.

duration than SPMS ($n = 34$) and PPMS ($n = 25$) patients (Table 2). The proportions of patients with North African ancestry did not differ between these three MS groups. Vitamin D levels were significantly higher in patients with RRMS compared with SPMS ($P = 0.0054$) and PPMS ($P = 0.0138$). To test for potential confounding factors vitamin D levels were assessed in multivariate analyses adjusted for age, ethnicity, gender, disease duration and season (exposure to sunlight). Vitamin D levels were independently and significantly higher in RRMS patients compared with patients with progressive forms of MS ($P = 0.049$).

Overall, a negative correlation between vitamin D levels and EDSS score was observed ($P = 0.0001$, $r = -0.33$), in association with a correlation between EDSS and MS type. Patients with impaired walking (defined by an EDSS ≥ 4) were likely to have reduced exposure to sunlight, which potentially explains the difference in mean vitamin D levels between patients with RRMS and those with progressive forms of MS. A linear model was thus constructed to study, separately, patients with an EDSS <4 and patients with an EDSS ≥4. Other factors associated with an EDSS <4 or >4 were first searched for using a bivariate model showing significant differences for age, vitamin D levels, disease duration and MS type, but not for gender, ethnicity and season (Table 3). As an example, patients aged <45 years were 5.75 times more likely to have an EDSS <4 [$P < 0.000001$, 95% confidence interval (CI) 3.03–10.93]. Also, patients with >20 ng/ml of vitamin D were 2.78 times more likely to have an EDSS <4 ($P = 0.0011$, 95% CI 1.49–5.00).

Secondly, age, vitamin D level, disease duration and MS type were included in a multivariate model and the correlation between EDSS and vitamin D levels was still significant after adjustment ($P = 0.041$, Table 3). Odds ratios showed that patients with vitamin D levels >20 ng/ml were 2.5 times more likely to have an EDSS <4 (95% CI 1.04–5.88).

As patients with RRMS had significantly lower mean EDSS scores than PPMS or SPMS patients, RRMS ($n = 122$) was specifically studied to refine the analysis of interactions between EDSS and vitamin D levels. A linear model was constructed using the same EDSS scores, i.e. EDSS <4 and ≥4. There was only a negative correlation between vitamin D levels and EDSS scores for patients with an EDSS <4 ($P = 0.0023$). In multivariate analyses, to rule out confounders (i.e. adjusted for age, gender, ethnicity, season and disease duration), the influence of vitamin D reached a significance threshold (odds ratio 2.56, 95% CI 1.00–6.67, $P = 0.05$).

Further analyses of correlations between vitamin D levels and EDSS scores for the whole study population showed that vitamin D levels were only correlated with EDSS score for patients with preserved walking ability, i.e. EDSS <4 ($P = 0.0012$, Fig. 1), but not for patients with EDSS >4 (Table 3). In order to minimize the role of disease duration on disability, the MSSS, an algorithm that relates EDSS scores to the distribution of disabilities in patients who have comparable disease durations, was considered [17]. It was found that in patients with RRMS and an EDSS <4, MSSS scores were significantly higher in patients with low vitamin D levels (<30 ng/ml) than in patients with normal vitamin D levels (2.51 ± 2.04 vs. 1.39 ± 1.78, $P = 0.0048$). This was not the case for RRMS patients with an EDSS ≥4 (5.13 ± 1.51 vs. 5.75 ± 1.98), but this subgroup was small ($n = 4$).

For patients with RRMS, the association between vitamin D levels and disability could also be influenced by other potentially confounding factors, such as relapse frequency and delay between the first and second relapse. Annualized relapse rate (ARR) was thus categorized into three groups: ARR > 1, ARR < 0.5 or ARR between 0.5 and 1. Vitamin D levels did not differ between these groups. The delay between the first relapse and a second relapse was also subdivided into three groups: <1 year, >1 year and

Table 2 Comparison of characteristics of patients with different subtypes of multiple sclerosis (MS)

	RRMS	SPMS	PPMS	*P* value
n (%)	122 (67.4)	34 (18.8)	25 (13.8)	
Gender ratio (F:M)	3.69 (96:26)	1.61 (21:13)	1.27 (14:11)	0.0146
Age (years)[a]	40.9 ± 12.1	54.3 ± 10.5	54.8 ± 9.7	0.0001
Ethnicity (E/NE)	116/5	31/3	23/2	0.4776
Disease duration (years)[a]	10.2 ± 8.2	19.9 ± 10.3	10.8 ± 7.3	0.0001
EDSS[a]	2.4 ± 1.9	6.2 ± 0.9	5.3 ± 1.5	0.0001
Vitamin D level (ng/ml)[a]	22.1 ± 11.2	15.6 ± 14.3	15.8 ± 8.9	0.003

RRMS, relapsing–remitting MS; SPMS, secondary progressive MS; PPMS, primary progressive MS; F:M, female:male; E/NE, European/non-European; EDSS, Expanded Disability Status Scale. [a]Values are mean ± SD.

Table 3 Bivariate and multivariate models according to Expanded Disability Status Scale score (below and above 4)

		Bivariate model			Multivariate model		
Covariable	Compared conditions	Global *P* value	*P* value comparison	OR (CI 95%)	Global *P* value	*P* value comparison	OR (CI 95%)
Ethnicity	E	0.54		1.5 (0.41–5.51)	0.74		1.4 (0.18–10.66)
Age (years)	≤45	0.000001		5.75 (3.03–10.93)	0.027		2.72 (1.12–6.6)
Vitamin D	>20 ng/ml	0.0012		2.78 (1.49–5.00)	0.041		2.5 (1.04–5.88)
Season		0.064			0.15		
	Autumn		0.017	2.95 (1.21–7.18)		0.23	2.13 (0.63–7.22)
	Autumn		0.53	1.29 (0.58–2.86)		0.63	0.76 (0.25–2.33)
	Autumn		0.084	2.18 (0.9–5.29)		0.15	2.54 (0.71–9.1)
	Winter		0.051	0.44 (0.19–1)		0.086	0.36 (0.11–1.16)
	Winter		0.52	0.74 (0.3–1.85)		0.79	1.19 (0.32–4.5)
	Spring		0.21	1.69 (0.74–3.87)		0.055	3.34 (0.97–11.43)
Gender	Female	0.11		1.72 (0.88–3.37)	0.61		1.29 (0.48–3.46)
MS type	RR		0.00001	16.67 (4.76–50)		0.0014	11.11 (2.5–50)
Duration	<10 years	0.00004		3.65 (1.97–6.77)	0.00496		3.41 (1.45–8.02)

OR, odds ratio; CI, confidence interval; E, European; NE, non-European; MS, multiple sclerosis; RR, relapsing–remitting; PP, primary progressive MS; SP, secondary progressive.

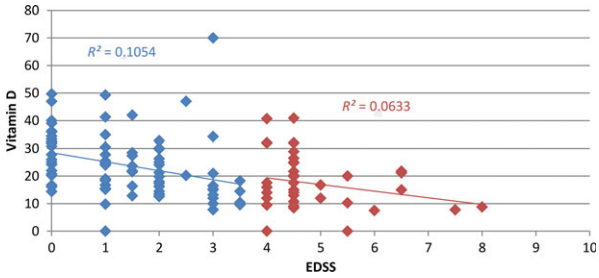

Figure 1 Correlation between Expanded Disability Status Scale (EDSS) and plasma vitamin D levels in fully ambulatory relapsing–remitting multiple sclerosis patients (EDSS < 4) compared with patients with reduced walking (EDSS ≥ 4). The correlation coefficient *R* is indicated for each EDSS range.

<2 years, and >2 years. Once again no significant differences were noted.

Discussion

Vitamin D deficiency is an important epidemiological risk factor for MS and is associated with a more active disease [8–10,19,20]. Herein, the influence of vitamin D levels on neurological disabilities and several parameters of this disease is reported. The clinical and historical characteristics of our cohort of 181 MS patients are in accordance with the literature [21]. Compared with patients with SPMS or PPMS, those with RRMS were more often female and had a younger age, a shorter duration of MS and a lower EDSS score. This study shows that patients with RRMS had higher vitamin D levels than patients with progressive forms of MS and, secondly, that for the whole cohort EDSS and vitamin D levels were negatively correlated. However, this correlation only included patients with a mild disability (EDSS < 4) when vitamin D acted as an independent factor (as assessed by multivariate analyses).

Vitamin D levels were correlated with EDSS in our whole cohort as well as in the subgroup of patients with RRMS. Some authors have suggested that vitamin D is associated with the level of disability caused by MS, and have suggested that vitamin D plasma levels may be predictive of a high EDSS score [9]. However, because patients with high EDSS scores are also more likely to remain indoors (thus reducing exposure to sunlight), it has been objected that a direct link between low vitamin D levels and the development of a disability cannot be ascertained [22]. Thus, this is why this important issue has been addressed in this study. Patients were separated into two groups: with and without a walking disability, i.e. patients with EDSS <4 and patients with EDSS ≥4. A significant correlation between vitamin D and EDSS

scores of <4 was ascertained and confirmed in multivariate analyses. This is an important point, as these patients are not likely to have reduced exposure to sunlight. It contrasts with a previous study indicating no or at best a weak negative association amongst patients with a low EDSS [10]. Moreover, this correlation remained significant after adjustment for disease duration using the MSSS, a scale that integrates disease duration into the assessment of neurological disability. This suggests that vitamin D deficiency could be associated with more rapid acquisition of neurological disabilities in MS.

In contrast, the correlation between vitamin D and EDSS no longer occurred for patients who had developed a walking disability (EDSS > 4), suggesting that, beyond this stage, the level of reduction in mobility does not influence vitamin D levels. This is illustrated by the fact that patients with a severe walking disability (high EDSS score >6), although more likely to have significantly reduced exposure to sunlight compared with patients with an EDSS score of between ≥4 and ≤6, did not have significantly lower vitamin D levels (data not shown). Subsequently, our data suggest that low vitamin D levels are related to disability in MS patients before a reduction in mobility, independently of disease type, duration, gender and age.

Similar results were obtained for the subgroup of patients with RRMS. In this subgroup, the risk for any disability (EDSS > 0) was increased by more than six-fold (data not shown) if vitamin D levels were <30 ng/ml. Additionally, the MSSS score, which takes into account the time to reach a given EDSS score, was also negatively correlated with vitamin D levels. This is an important point, as it is has been shown that vitamin D levels decrease with age.

There are several factors that have been associated with disability in patients with RRMS [21]. For many years, a high ARR and a short time between the first two relapses have been described as two essential factors associated with a higher risk of rapid progression to disability. In our group of RRMS patients, however, there was no correlation between these parameters for MS activity and vitamin D levels. This may suggest that the influence of vitamin D levels on disability is at least partly independent of the inflammatory process. In several neurological conditions, low vitamin D levels are associated with increased severity of the neuronal insult, in both neurodegenerative processes and vascular disorders. It has been shown recently that amyotrophic lateral sclerosis evolved more than four-fold less rapidly in patients with normal vitamin D levels [15]. Additionally, vitamin D enhanced the role of neurotrophic factors in motoneuron survival and completely blocked fas-induced cell death *in vitro* [15]. However, the variety of disorders in which vitamin D levels have been described as a prognosis modulator favour a non-specific mechanism, and an indirect role through the activity of neurotrophic factors enhances this hypothesis.

In our cohort, although there were a smaller number of patients with North African ancestry, mean vitamin D level was significantly lower than that of Europeans. A previous MS cohort has already underlined the more severe evolution of this disease in patients with North African ancestry [23]. This similar finding in our MS cohort further agrees with the hypothesis of a deleterious role for vitamin D deficiency in neuronal repair or survival. A causal role for vitamin D in disability progression could not be tested in this study due to unmeasured subclinical activity (e.g. atrophy) and lack of prospective follow-up. This will be an important challenge for future randomized controlled trials.

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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