Vitamin D metabolic pathway alterations and risk of multiple sclerosis in clinically isolated syndromes

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Introduction and purpose

- Experimental and clinical observations in fact provide growing evidence that vitamin D is one of the key environmental factors exerting significant influence over immunoregulation, and ultimately affecting multiple sclerosis (MS) prevalence (1).
- Even though circulating 25(OH)D concentrations are predominantly influenced by ultraviolet B exposure from sunlight, evidence suggests that concurrent treatments, genetic and epigenetic factors are also important in the control of 25(OH)D levels (2)
- The aim of the present study is to assess the presence of vitamin D metabolic pathways alterations related to MS risk

Methods

- Inclusion criteria:
  - Patients admitted to our hospital between January 1, 2000 and December 31, 2008 for first time monophasic neurologic symptoms not attributable to other diseases;
  - age 18-55 years;
  - syndrome onset within 3 months of clinical, blood samples, CSF, and MRI examinations;
  - availability of a serum sample obtained at the moment of the hospitalization and stored at -20°C since then.
- Comprehensive demographics, previous history and treatment ongoing at the moment of the admission, clinical data of the first neurological episode, follow up information and conversion to CDMS were obtained from hospital charts and confirmed by the patients. Baseline MRIs were also obtained.
- Vitamin D methabolites levels have been assessed using DEQAS validated SPE-HPLC_MS/MS. The method is based on direct analysis of serum by an automated platform involving on-line coupling of a solid-phase extraction (SPE) workstation to a liquid chromatograph–tandem mass spectrometer (HPLC-MS/MS). The analyses were performed by bidimensional reversed-phase LC (RP-LC) separation followed by electrospray ionization in positive mode (ESI+) and MS/MS detection. The analytical columns were a C18 and a PFP (3).
- Parametric or nonparametric comparative statistics were performed according to the normality of the distributions of the continuous variables. χ^2 test was performed to compare categorical variables. Seasonally adjusted values of vitamin D metabolites were used for analysis. K-means clustering was used to assess the presence of different patterns of vitamin D metabolites levels, and Cox proportional hazard models with restricted cubic splined were used to study the association between different clusters and hazard of MS.

Results

Patient demographics and conversion rate to clinically definite multiple sclerosis (CDMS). Mean age of the patients was 33,2 ± 8,6 years, 40 were males and 80 females (ratio 1:2). Sixty four 64 patients (53.3 %) developed CDMS: 24 patients (20.0 %) within the first year, 38 (31.7 %) within the second year. Of the 92 patients who had at least 5 years of follow-up, 56 (60.9 %) had converted to CDMS.

Results

Table 1. Baseline characteriistics of patients according do clusters pattern of vitamin D metabolites levels

	25-hydroxyvitamin D levels[a]					
	< 25th percentile		25th-75th percentile		> 75th percentile	
	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 1	Cluster 2
Cholecalciferol levels, mean nmol/l ± SD [a]	5.2 ± 3.3	3.5 ± 6.2	7.7 ± 3.9	5.7 ± 2.5 ‡	7.9 ± 2.9	12.8 ± 9.5
25(OH)D levels, mean nmol/l ± SD [a]	17.0 ± 3.6	20.1 ± 4.0	75.1 ± 3.8	43.0 ± 4.3	67.6 ± 7.2	55.0 ± 8.1
3-epi-25(OH)D levels, mean nmol/l ± SD [a]	0.3 ± 0.2	0.9 ± 0.7	1.1 ± 0.3	0.6 ± 0.3 ‡	7.89 ± 2.9	1.5 ± 0.6 *
24,25(OH)D levels, mean nmol/l ± SD [a]	2.1 ± 0.4	2.7 ± 0.7 †	4.0 ± 0.8	2.6 ± 0.6 ‡	5.2 ± 1.0	5.8 ± 1.5
1,25(OH)D levels, mean pmol/l ± SD [a]	153.1 ± 15.5	112.5 ± 16.3 ‡	177.3 ± 16.7	100.1 ± 12.4 ‡	136.6. ± 10.9	188.5 ± 15.6 ‡
Age at blood collection, mean yr ± SD	33.8 ± 10.4	29.1 ± 6.2	31.5 ± 6.2	31.7 ± 8.8	32.7 ± 11.1	33.4 ± 9.0
Gender						
Females, No. (%) [b]	17 (77.3)	4 (50.0) ¶	17 (63.0)	22 (66.7)	7 (63.6)	13 (68.4)
Males, No. (%) [b]	5 (22.7)	4 (50.0)	10 (37.0)	11 (33.3)	4 (36.4)	6 (31.6)
Type of onset, No. (%) [b]						
monofocal	21 (95.5)	6 (75.0)	25 (92.6)	25 (75.8)	11 (100)	17 (89.5)
multifocal	1 (4.5)	2 (25.0)	2 (7.4)	8 (24.2)	0 (0.0)	2 (10.5)
T2 lesions at brain MRI, No. (%) [b]						
0-1	2 (9.1)	3 (37.5)	3 (11.1)	6 (18.2)	0 (0.0)	3 (15.8)
2-9	8 (36.4)	2 (25.0)	20 (74.1)	16 (48.5)	6 (54.5)	10 (52.6)
> 9	12 (54.5)	3 (37.5)	4 (14.8)	11 (33.3)	5 (45.5)	6 (31.6)
Presence of Gd-enhancing lesions, No. (%) [b]	9 (40.9)	2 (25.0)	8 (29.6)	18 (54.5)	5 (45.5)	7 (36.8)
Presence of CSF oligoclonal bands, No. (%) [b]	15 (68.2)	6 (75.0)	20 (74.1)	27 (81.8)	5 (45.5)	14 (73.7)

[a] Adjusted for season of blood draw and corticosteroids therapy (converted in prednisone equivalent doses) in the 4 weeks prior to blood draw.
[b] Percentages of cluster total
¶ $p < 0.10$
* $p < 0.05$
† † $p < 0.01$
‡ ‡ $p < 0.001$

Figure 1. Smoothed plot of log relative hazard of CDMS risk according to cluster pattern of vitamin D metabolites levels in patients with low (A), medium (B) or high © 25-hydroxyvitamin D

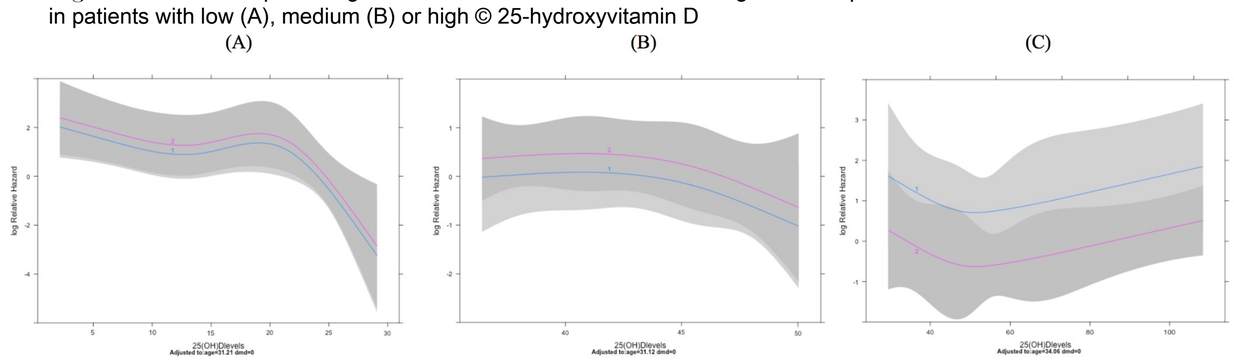

Table 2. Hazard ratios with 95% Cis of CDMS risk according to clusters pattern of vitamin D metabolites levels

	Vitamin D Metabolites Levels		
	< 25th percentile	25th-75th percentile	> 75th percentile
Cluster 1	reference	reference	reference
Cluster 2	0.92 (0.33-2.54)	1.87 (0.90-4.01)*	0.26 (0.07-0.98) †

[1] Hazard ratios were estimated by Cox proportional hazards regression. Multivariable models were adjusted for age, gender and use of disease modifying therapies before a second clinical event.
* $p < 0.10$
† † $p < 0.05$
‡ ‡ $p < 0.01$

Conclusions

- We have confirmed in this study that high 25(OH)D levels are associated with a lower risk of MS (4).
- Patients within the same range of 25(OH)D levels may have not the same risk of disease. In fact, among patients with medium to high 25(OH)D levels, different cluster patterns of vitamin D metabolites have different hazard of disease, where clusters with the highest conversion rate of 25(OH)D to the active form 1,25(OH)2D have the lowest risk of conversion to MS.
- Differences in the metabolism of vitamin D exist and cause 25(OH)D measurement to be uninformative per se. Particularly, 25(OH)D levels are not representative in the single patient of the final active form of vitamin D, 1,25(OH)2D, as these depend on the activity of catabolic enzymes (CYP24) and activating enzymes (CYP271b), whose activity could be altered in MS patients.

References

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