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Serum 25-hydroxyvitamin D levels in multiple sclerosis patients from the north of Portugal

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ABSTRACT

Increasing evidence has shown that individuals with Multiple Sclerosis (MS) have lower 25-hydroxyvitamin D [25(OH)D] levels compared to healthy controls. There is no information regarding 25(OH)D levels and MS in Portugal. Therefore the aim of the current study was to examine the levels of 25(OH)D in a group of patients with MS and in healthy matched controls, as well as the association of 25(OH)D levels with disease course, disability and severity. A group of 244 unrelated Portuguese patients, with a definitive diagnosis of MS, and 198 ethnically matched healthy controls were included in the study. A sub-group of patients with recent disease onset was included. Serum 25(OH)D was measured using an electrochemiluminescence binding assay.

The mean serum level of 25(OH)D in patients with MS was 39.9 ± 22.0 nmol/L, which was significantly lower ($p < 0.0001$) than those in healthy controls, 55.4 ± 23.4 nmol/L. There was a negative correlation between 25(OH)D levels and EDSS ($r = -0.293$, $p < 0.0001$) and MSSS scores ($r = -0.293$, $p < 0.0001$). In multiple logistic regression analysis adjusted for age, gender, disease form, EDSS, disease duration and MSSS, 25(OH)D levels were independently associated with EDSS ($p = 0.004$) and disease duration ($p = 0.016$), and with MSSS ($p = 0.001$).

In accordance with the majority of the literature, low serum 25(OH)D levels were associated with susceptibility and disability in MS patients from Portugal. Lower serum 25(OH)D levels were also found in patients with a recent disease onset, supporting vitamin D levels as a risk factor for MS.

1. Introduction

Multiple Sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) in young adults. The cause of MS remains poorly understood, but it is widely believed to be an autoimmune disease occurring in genetically susceptible individuals after exposure to as-yet undefined environmental factors [1].

Among non-infectious environmental factors, there is a recent increase in studies investigating vitamin D levels in MS pathogenesis. Vitamin D is the main regulator of calcium and phosphorus levels in the body, and deficiency is associated with rickets in children, and with osteomalacia and osteoporosis in adults. Experimental studies have shown that vitamin D has a potent immunomodulatory activity [2], significantly affecting the regulation of immune responses, restoring beneficial proportions of the populations of Th2 and Th1 lymphocytes, with the overall effect of attenuating inflammatory reactions [3,4]. Also, vitamin D supplementation resulted in multiple beneficial immunological effects: in particular, with stimulation of Tregs [5] and of the favorable IL-10 cytokine [6,7], diminution of the pro-inflammatory Th17 lymphocytes [8] and the deleterious cytokine IL-17 [9], and attenuation of B-cell immunoreactivity [10].

A substantial evidence base now exists to support an association between vitamin D deficiency and low levels of its metabolite 25-hydroxyvitamin D3 [25(OH)D] in the onset and development of MS. Since

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Table 1
Summary of the case-control studies that studied the influence of vitamin D levels in MS susceptibility since 2010.

Study	Country	Study design	Sample size (MS/Control)	Season	25 (OH)D levels (nmol/L)		
					MS	Control	p
Shaygannejad [11]	Iran	Case-control	(50/50)	NA	48.0	62.0	0.036
Lonergan [12]	Ireland	Case-control	(329/226)	Winter	38.6	36.4	n.s.
Gelfand [13]	USA	Case-control	(339/342)	NA	29.7	36.6	0.0001
Hatamian [14]	Iran	Case-control	(52/52)	NA	66.1	92.6	0.003
Kirbas [15]	Turkey	Case-control	(30/30)	NA	67.9	106.3	0.001
Mazdeh [16]	Iran	Case-control	(75/100)	Winter	NA	NA	0.003
				Summer	29.4	58.5	
Shahbeigi [17]	Iran	Case-control	(98/17)	Summer	79.0	89.3	0.047
Hejazi [18]	Iran	Case-control	(37/37)	Winter	20.7	15.8	n.s.
Niino [19]	Japan	Case-control	(70/40)	Winter	42.7	49.9	< 0.05
Behrens [20]	Germany	Case-control	(76/76)	All year	NA	NA	0.002
Karampoor [21]	Iran	Case-control	(1000/700)	Winter	36.2	64.4	NA
Becker [22]	Brazil	Case-control	(67/61)	Winter	58.0	67.7	0.957
				Summer	74.8	77.3	0.115
Brola [23]	Poland	Case-control	(184/78)	Winter	33.4	36.4	0.012
				Summer	60.6	62.6	0.256
Yamout [24]	Lebanon	Case-control	(50/99)	All year	53.9	36.2	0.002
Zhang [25]	China	Case-control	(141/282)	Winter Summer	39.7	51.4	< 0.0001
Bettencourt	Portugal	Case-control	(244/198)	Winter	34.5	42.2	0.0003
				Summer	48.7	68.2	< 0.0001

2010 several studies have addressed the influence of vitamin D levels in disease susceptibility [11–25] (Table 1). In 2014, a meta-analysis of previous studies concluded that low vitamin D levels are associated with an increased risk of MS [26]. In a large prospective study, published in 2006, Munger et al. found that the risk of MS decreased with increasing of serum levels of 25-hydroxyvitamin D [27]. Also, two other studies showed evidence for possible neuroprotection of vitamin D in clinically isolated syndrome [28,29]. Nevertheless it is less clear whether vitamin D has a role in MS progression.

Regarding disease course, lower levels of 25(OH)D were found in secondary-progressive (SP) MS when compared to relapsing-remitting (RR) MS [30]. In a retrospective longitudinal study, Muris et al. [31] assessed whether the vitamin D status in RRMS patients is associated with the time of conversion to SPMS and found an association between low vitamin D status at the start of RRMS and the early conversion to SPMS. Lower 25(OH)D levels in patients with RRMS have been associated with higher clinical and radiographic disease activity [23,32–34] and with the degree of disability in fully ambulatory RR patients [35].

There is no data regarding vitamin D levels in MS patients in Portugal, therefore the aim of the current study was to examine the levels of 25(OH)D in a population-based group of patients with MS and in healthy matched controls, as well as the association of 25(OH)D levels with disease course, disability and severity.

2. Subjects and methods

2.1. Patients and controls

From a total of 632 unrelated Portuguese patients with a definitive diagnosis of MS, according to the revised McDonald criteria [36], recruited from the neurology outpatient clinic of Centro Hospitalar do Porto – Hospital de Santo António (HSA) a subgroup of 244 patients, that had 25(OH)D levels measured before supplementation, were included. The Expanded Disability Status Scale (EDSS) [37] and Multiple Sclerosis Severity Scale (MSSS) [38] were used to measure, respectively, physical disability and disease severity. The control group comprised 198 ethnically matched healthy controls (HC) and their evaluation was described previously [39]. A sub-group of recently diagnosed (2012–2015) patients (n = 74), in which Vitamin D levels were measured at diagnosis, was studied independently. Exclusion criteria comprised of individuals (cases or controls) with disorders related to vitamin D deficiency such as rickets or parathyroid pathologies or receiving vitamin D therapy in the 3 months preceding data collection; patients with other neurological or immune-mediated disease; those with skin diseases or medication use with a medical recommendation to avoid exposure to the sun; and patients having experienced a relapse in the last 30 days. This study was approved by the hospital Medical Ethical Committee and written informed consent was obtained from all participants.

2.2. 25(OH)D measurement

Blood was collected in Vacuette® Z Serum Separator Clot Activator tubes. Serum was obtained by centrifugation and stored in several aliquots at −20 °C until analyzed. Serum total 25(OH)D was chosen as a reliable marker of individual vitamin D status as it reflects vitamin D obtained from food sources and cutaneous synthesis, and it is not prone to diurnal variation. Serum 25(OH)D was measured using an electrochemiluminescence binding assay (ECLIA) for the *invitro* determination of total 25-hydroxyvitamin D (Elecsys® Vitamin D total, Cobas, Roche©), measurement range: 7.50–175 nmol/L.

Much debate has taken place over the definition of vitamin D deficiency, nevertheless a 25(OH)D concentration < 50 nmol/L (20 ng/ml) is currently considered an indication of vitamin D deficiency, whereas a 25(OH)D concentration of 50–75 nmol/L (20–30 ng/ml), is considered to indicate insufficiency; concentrations > 75 nmol/L (30 ng/ml), are considered to be adequate [40–43].

2.3. Statistical analysis

Continuous data were checked for normality using the Kolmogorov-Smirnov test. Differences between groups were tested using the Mann-Whitney *U* test and Kruskal-Wallis test. Spearman's correlation coefficients were calculated to test interactions between continuous variables.

Multivariate linear regression was used to test the association of 25(OH)D levels with EDSS and MSSS, adjusting for age, gender and disease course; and multivariate logistic regression was used to test the association of 25 (OHD levels with MS status adjusting for age and gender.

A *p*-value below 0.05 was considered to be statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences software (version 23, IBM SPSS Statistics, NY, USA).