

# No correlation was observed between vitamin D levels and disability of patients with multiple sclerosis between latitudes 18° and 30° South

Não foi observada correlação entre os níveis séricos de vitamina D e a incapacidade em pacientes com esclerose múltipla entre as latitudes Sul 18° e 30°

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## ABSTRACT

**Objective:** Vitamin D has taken center stage in research and treatment of multiple sclerosis (MS). The objective of the present study was to assess the serum vitamin D levels of a large population of patients with MS and controls living in a restricted tropical area. **Methods:** Data from 535 patients with MS and 350 control subjects were obtained from 14 cities around the Tropic of Capricorn. **Results:** The mean serum 25-OH vitamin D level was  $26.07 \pm 10.27$  ng/mL for the control subjects, and  $28.03 \pm 12.19$  ng/mL for patients with MS. No correlation was observed between vitamin D levels and the disability of patients over the disease duration. **Conclusion:** At least for the region around the Tropic of Capricorn, serum levels of vitamin D typically are within the range of 20 to 30 ng/mL for controls and patients with MS.

**Keywords:** vitamin D; multiple sclerosis; latitude.

## RESUMO

**Objetivo:** Vitamina D assumiu um papel central na pesquisa e tratamento da esclerose múltipla (EM). O objetivo deste estudo foi avaliar os níveis séricos de vitamina D de pacientes com EM e controles que residem em uma área tropical. **Métodos:** Foram analisados dados de 535 pacientes com EM e 350 indivíduos controle em 14 cidades próximas ao Trópico de Capricórnio. **Resultados:** O valor médio da determinação de 25-OH vitamina D foi  $26,07 \pm 10,27$  ng/mL para controles e  $28,03 \pm 12,19$  ng/mL para pacientes com EM. Não houve correlação entre os níveis de vitamina D e o grau de incapacidade ao longo da duração da doença. **Conclusão:** Pelo menos na região que cerca o Trópico de Capricórnio, os níveis séricos de vitamina D estão entre valores de 20 a 30 ng/mL tanto para controles quanto para pacientes com EM.

**Palavras-chave:** vitamina D; esclerose múltipla; latitude.

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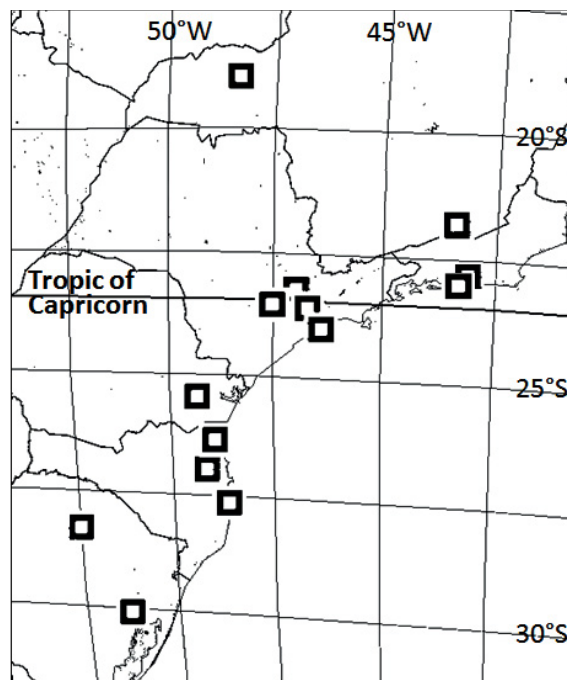
There is no doubt that vitamin D has many biological effects on the human body. The potential effects of this hormone on bone metabolism, immunological responses and degenerative diseases has led to a dramatic surge of vitamin D testing in the fields of health and disease<sup>1</sup>. Within multiple sclerosis (MS), a disease with marked predominance in areas of high latitude and long winters, serum assessment and supplementation of vitamin D rapidly took on great importance with regard to evaluating and treating MS patients. Nonetheless, while there is mounting evidence that vitamin D exerts broad regulatory effects on cells of the adaptive and innate immune systems<sup>2,3</sup>, there is remarkably little evidence that treatment with vitamin D can influence MS evolution and prognosis<sup>4,5,6,7</sup>. So far, no clinical trial has provided any clear evidence that vitamin D alone can alter the course of an extremely complex disease like MS. Other vitamins<sup>8,9,10</sup>, genetic predisposition<sup>11,12</sup> and several environmental factors<sup>13,14,15,16</sup> may also play a substantial role in MS.

However strong the role of vitamin D in the orchestra of factors associated with MS may be, this might be better seen in high-latitude countries. The main source of vitamin D is through endogenous production, whereby the ultraviolet rays in sunlight irradiate the 7-dehydrocholesterol present in the skin, to generate cholecalciferol<sup>17</sup>, which is subsequently activated in the liver and kidney. Therefore, vitamin D levels may be higher in areas of the world where individuals have more exposure to sunlight, such as the tropics. The present study aimed to assess the potential effects of vitamin D levels on the development and progression of disability due to MS in an area at latitudes around the Tropic of Capricorn.

## METHODS

The latitude of the Tropic of Capricorn is 23°27' S and the geographic area included in the present study ranged from latitudes 18° to 30° S. Only patients and control subjects living in this restricted area were included in the study. This is a tropical to subtropical zone, with long summers and mild winters. No snow has ever been detected in any of the cities in this study. The length of the day varies between 10.30 hours (southern winter solstice) and 15.30 hours (southern summer solstice). Rainfall typically varies between 1,000 and 1,500 millimeters per year in this area. The 14 cities participating in the study were Blumenau, Curitiba, Florianopolis, Joinville, Juiz de Fora, Jundiai, Niteroi, Passo Fundo, Porto Alegre, Rio de Janeiro, Santos, Sao Paulo, Sorocaba, and Uberlandia. Their locations are shown on the map in Figure 1.

This study was approved by the Ethics Committee. Neurologists from different cities within the established latitude levels collected data from their patients with MS and from control subjects of similar age and gender distribution. Patients were included in the MS group if they fulfilled the McDonald's criteria revised in 2005<sup>18</sup>. Patients and control



**Figure 1.** Schematic map of the location of the participant cities and their geographical information in relation to the Tropic of Capricorn (Blumenau, Curitiba, Florianopolis, Joinville, Juiz de Fora, Jundiai, Niteroi, Passo Fundo, Porto Alegre, Rio de Janeiro, Santos, Sao Paulo, Sorocaba, and Uberlandia).

subjects were only included in the study if they had not been taking vitamins (vitamin D or multivitamin compounds) as supplements for at least six months. Smoking habits were considered positive if the patient or control subject had smoked within the previous three years, independently of smoking at the time of blood collection for vitamin D assessment. No dietary restrictions were imposed on the subjects of this study, except for fasting for at least eight hours at the time of blood sampling. The 25-OH vitamin D levels were detected by means of chemiluminescent immunoassay. Although each participant's serum level was assessed in a clinical laboratory in his or her own city, the method used for this assessment was uniform.

Statistical analyses were performed blindly by one of the authors not involved in other phases of the project (PNS). The data were analyzed using GraphPad Prism, using two-way and one-way ANOVA, Spearman correlation, Kruskal-Wallis test, Mann-Whitney test and Student's t test. Significant values were considered to be those with  $p < 0.05$ .

## RESULTS

Data were obtained from 350 controls (22% males, median age 36 years, smokers = 7.4%) and 535 patients with MS (25.9% males, median age 35 years, smokers = 14%). The mean serum 25-OH vitamin D level was  $26.07 \pm 10.27$  ng/ml

for the control subjects and  $28.03 \pm 12.19$  ng/ml for patients with MS. Although the difference was considered to be significant through direct comparison using Student's t test ( $p = 0.01$ ), the levels both for healthy subjects and for patients were within the range of 20–30 ng/ml, which can be considered to be low, but without characterizing vitamin D deficiency. Analysis on the patients and controls for values  $< 20$  ng/ml or  $> 30$  ng/ml did not show any significant difference between the groups ( $p = 0.10$ ). The same was observed for all the data in this study, such that the majority of the participants systematically presented with serum vitamin D levels within the range of 20–30 ng/ml for all the parameters assessed (gender, age, MS variables, etc.). Fewer than 15% of the patients and controls showed serum vitamin D levels

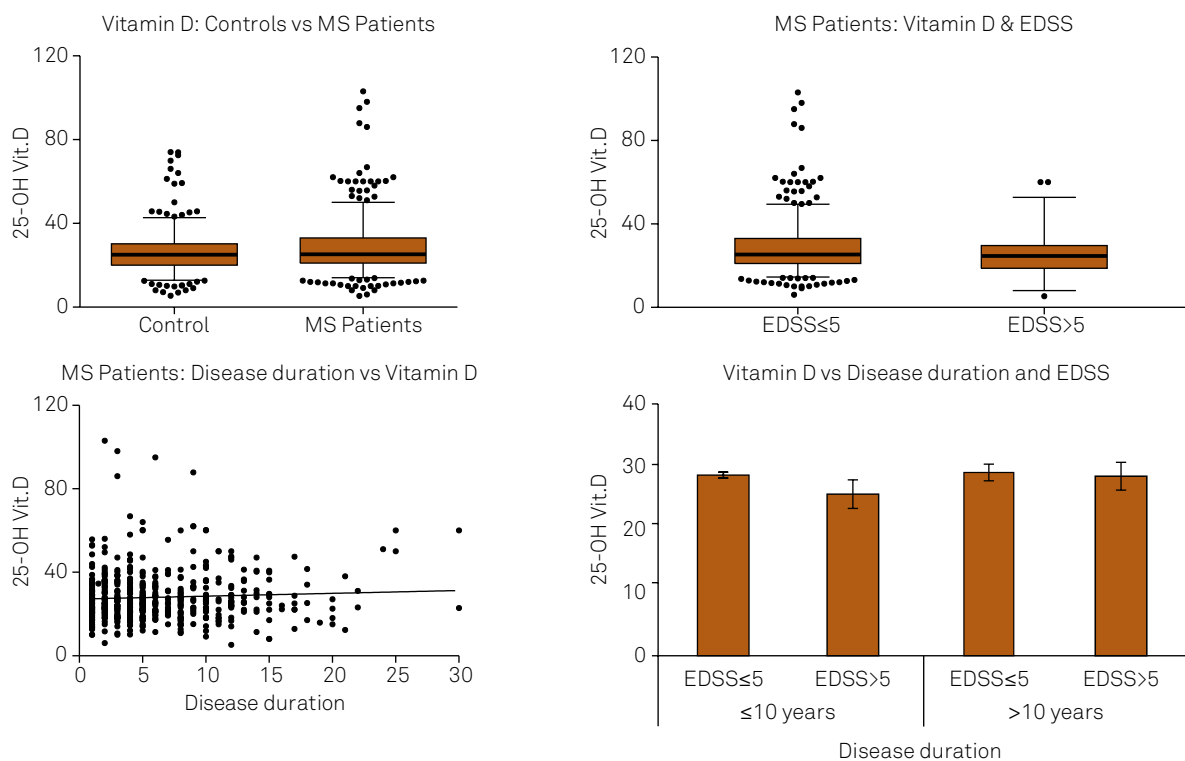
$< 20$  ng/ml. A summary of the data is presented in the Table and Figure 2.

No correlation was found between smoking and vitamin D levels among either the controls ( $p = 0.87$ ) or the patients ( $p = 0.74$ ). Through taking age to be a factor with the potential to modify vitamin levels, it was observed that, for the controls, there was a negative correlation between age and serum 25(OH) vitamin D levels ( $p = 0.01$ ). The same was not observed for patients, for whom age did not seem to affect serum vitamin D levels ( $p = 0.3$ ). However, once again, even though the serum vitamin D levels were significantly different, the patients and controls typically showed levels within the range of 20–30 ng/ml.

**Table.** Vitamin D levels in serum of patients with multiple sclerosis and control subjects.

Variable	Population	Serum vitamin D (ng/ml) Average plasma levels	Smoking habits		EDSS	
			Smokers	Non-smokers	$\leq 5.0$ (n = 480)	$\geq 5.5$ (n = 55)
Patients with MS	n = 535	$28.03 \pm 12.19$	$28.10 \pm 12.42$	$27.61 \pm 10.84$	$28.18 \pm 12.14$	$26.69 \pm 12.67$
	M = 139					
	F = 396					
	med age = 35					
Control subjects	n = 350	$26.07 \pm 10.27$	$26.09 \pm 10.14$	$25.77 \pm 11.90$		
	M = 79					
	F = 271					
	med age = 36					

MS: multiple sclerosis; M: males; F: females; med age: median age in years; EDSS: expanded disability scale score.



**Figure 2.** Levels of serum 25(OH) vitamin D in control subjects (n = 350) and patients with multiple sclerosis (n = 535). Average and median levels of vitamin D are similar for both groups and within the 20–30 ng/ml. According to different sources, this level is considered to be low, but not insufficient; while other sources state this level is normal. No correlation was observed between levels of vitamin D and disability over time.

Men showed significantly higher vitamin D levels than women, among the patients ( $p = 0.005$ ), and borderline higher values among the controls ( $p = 0.06$ ). Again, serum vitamin D levels were mostly within the 20-30 ng/ml range for both genders and there was no difference between the groups regarding values  $< 20$  ng/ml or  $> 30$  ng/ml.

Disease duration ( $p = 0.20$ ) and disability ( $p = 0.39$ ) measured using the Expanded Disability Score Scale<sup>18</sup> (EDSS) did not show correlations with the serum vitamin D levels in patients with MS (Figure 2). Aggressiveness of the disease, measured as higher EDSS values with shorter disease duration, was not shown to differ between patients whose serum vitamin D levels were higher or lower in this study ( $p = 0.65$ ) (Figure 2).

## DISCUSSION

Sunlight is essential to life on Earth. Ultraviolet radiation, infrared and visible light are all part of the solar light spectrum, and around 90% of the radiation received on Earth arrives in the form of ultraviolet light<sup>19</sup>. Ultraviolet radiation influences vitamin D synthesis and it is beneficial for complex metabolic pathways of homeostasis in the human body. At the same time, ultraviolet radiation is a very important environmental hazard for human skin, and exposure needs to be restricted to a few hours a day. Early morning and late afternoon are the best times of the day for exposure to the sun in the tropics and many people take daily walks or do activities at these times. This is considered to be good for health, and is recommended by many health-care personnel.

Although the Endocrine Medical Society recommends vitamin D levels of at least 30 ng/ml<sup>20</sup>, the Food and Nutrition Board considers that serum vitamin D levels  $> 20$  ng/ml are adequate<sup>21</sup>. The vast majority of the participants in this study presented with levels  $> 20$  ng/ml.

The present study showed that there were no differences in the average vitamin D levels assessed, between healthy controls and patients with MS. These subjects were not supplementing their diets with vitamins and were continuously exposed to the mild climate at latitudes around the Tropic of Capricorn, between 18° and 30° South. Thus, exposure to the sun may have played an important part in maintaining uniform vitamin D levels among the participants of this study. However, the authors are aware that daily sun exposure was not a parameter assessed in this study, and this may be a limitation to interpretation of results. Nevertheless, the large number of patients and controls enrolled in this study may overcome the limitations of measuring daily sun exposure. In addition, results were not different in any of the cities or between control subjects and patients, which further suggests that sun exposure and vitamin D were average for these individuals.

It is important to remember that ultraviolet radiation has other effects beyond metabolism of vitamin D that might explain the beneficial effects of living in the tropics. Ultraviolet radiation induces cytokines with immunosuppressive behavior (CXCL12), affects the non-vitamin D pathway in dendritic cells, and induces production of the immunoregulatory molecule cis-urocanic acid<sup>22,23</sup>. An MRI study on people with MS showed that sun exposure, independently of vitamin D levels, was associated with better outcomes<sup>24</sup>. A study on the age of onset of MS did not show correlation with vitamin D levels<sup>25</sup>. A very recent multicenter study showed that, although potentially present, the relationship between vitamin D levels and the conversion from clinically isolated syndrome into clinically defined MS may be attenuated by other factors<sup>26</sup>. In fact, the Cochrane database states that “the current level of evidence for the effectiveness of vitamin D supplementation in the management of people with MS does not, at present, allow confident decision-making about the use of vitamin D in MS”<sup>27</sup>.

Again, in the region of the Tropic of Capricorn, Australian researchers have not found that vitamin D supplementation has any effect on patients with MS<sup>28</sup>. Serum vitamin D levels may have been a simplistic way to assess a very complex regulatory pathway in MS. No clinical trial has shown any success from vitamin D supplementation for controlling MS. Independently of vitamin D production and serum vitamin D levels, sunlight and particularly ultraviolet radiation may be responsible for combating disease incidence and progression, with a positive link with latitude. This has also been stated by other researchers<sup>29,30,31</sup>. Despite a few hundred systematic reviews and meta-analyses, there is no highly convincing evidence of any clear role for vitamin D in controlling any disease outcome<sup>32</sup>. In fact, vitamin D may be a surrogate marker for ill health, rather than the cause of diseases it is blamed for.

The results shown here may not be reproducible in studies carried out in the higher northern latitudes. There have been studies showing, for example, a correlation between EDSS and serum vitamin D levels in Europe and North America<sup>33,34</sup>. There have also been studies showing a potential role for serum vitamin D levels for MS relapse prevention in some populations<sup>35,36,37</sup>, and control over lesions seen on MRI in some countries<sup>38</sup>. The authors of the present study do not dispute the role of vitamin D in human health and the immune system. However, we question whether results from all parts of the world would be the same if vitamin D were the only parameter of importance in such a complex disease. It is important to highlight that many MS studies carried out in Latin America show results differing from those published by our peers at higher latitudes in the northern hemisphere. In Mexico, a country crossed by the Tropic of Cancer, vitamin D levels do not seem to differ between controls and MS patients either<sup>39,40</sup>. A recent Brazilian study of 67 patients with MS showed that,

although there was an association of low levels of vitamin D and MS relapses, no correlation between levels of vitamin D and disability could be observed<sup>41</sup>. In the Netherlands, similar results were found regarding the lack of clear association between levels of vitamin D and disability status in MS<sup>42</sup>.

Multiple sclerosis must not be seen as a simple disease, explained by deficiency of a particular vitamin, or having a uniform profile worldwide. At least for the latitudes of 18° to 30° South, serum vitamin D levels did not differ between

controls and MS patients, and were not related to worse disease evolution.

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