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The correlation between the level of 25-hydroxyvitamin D [25(OH)D] and residency of multiple sclerosis patients in Montenegro – higher levels only in men in the north of the country

Povezanost nivoa 25-hidroksivitamina D [25(OH)D] i mesta stanovanja bolesnika sa multiplom sklerozom u Crnoj Gori – viši nivoi samo kod muškaraca na severu zemlje

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Abstract

Background/Aim. Multiple Sclerosis (MS) is a chronic neurological disease associated with low serum levels of 25-hydroxyvitamin D [25(OH)D]. The aim of this study was to determine the association between serum levels of 25(OH)D and the latitude as well as clinical MS severity and progression expressed by expanded disability status scale (EDSS) and multiple sclerosis severity score (MSSS). **Methods.** A total of 196 patients, from North and South of Montenegro, aged 18 to 65 years, with confirmed diagnosis of MS were recruited for the study. Serum samples were collected for 25(OH)D measurement. Control group consisted of 196 health controls, randomly selected from medical staff employed in health centers from three cities in North and Clinical Centre of Montenegro from the South. **Results.** The serum levels of 25(OH)D were significantly lower in MS patients compared to controls ($p < 0.001$). The serum levels of 25(OH)D were significantly different in regard to gender, with women showing lower levels. Although in the entire group of patients there was no statistical correlation between the levels of 25(OH)D and their residence, the significantly higher levels of 25(OH)D were detected in men from the North compared to women. The course of the disease had an impact on the 25(OH)D serum levels. 25(OH)D levels also significantly correlated with clinical parameters of both, disability (Spearman's r = -0.23, p = 0.001) and progression (Spearman's r = -0.25, p = 0.0004) of MS. **Conclusion.** Serum levels of 25(OH)D were associated with disability and progression in MS patients. Lower levels of 25(OH)D were detected in female patients from the North. The low level of 25(OH)D cannot be solely explained with unfavorable latitude and insufficient sun exposure, therefore further genetic analysis is needed.

Key words:
multiple sclerosis; vitamin d; geography, medical; severity of illness index; disease progression; sex factors; montenegro.

Apstrakt

Uvod/Cilj. Multipla skleroza (MS) je hronična neurološka bolest, povezana s niskim serumskim nivoom 25-hidroksivitamina D [25 (OH)D]. Cilj rada bio je da se ispitaju povezanost nivoa 25(OH)D u serumu sa geografskom širinom mesta boravka obolelih i onesposobljenošću i progresijom MS, izraženim preko proširenog skora stepena onesposobljenost (EDSS) i stepena spasticiteta obolelih od multiple skleroze (MSSS). **Metode.** U studiju je bilo uključeno ukupno 196 bolesnika sa severa i juga Crne Gore, starosti između 18 i 65 godina, sa definitivnom dijagnozom MS. Od svakog pojedinačnog bolesnika je na dan prijema uziman serum za analizu nivoa 25(OH)D. Kontrolna grupa se sastojala od 196 zdravih ispitanika, koji su metodom slučajnog uzorka birani među medicinskim osobljem tri bolnice sa severa i Kliničkog centra Crne Gore, sa juga zemlje. **Rezultati.** Nivoi serumskog 25(OH)D bili su značajno niži kod bolesnika sa MS u odnosu na kontrolnu grupu ($p < 0.001$). Nivo 25(OH)D značajno se razlikovao u odnosu na pol, pri čemu su kod žena registrovani niži nivoi. Mada kod svih ispitanika nije zabeležena statistički značajna korelacija između nivoa 25(OH)D i prebivališta, nađen je značajno viši nivo 25(OH)D kod muškaraca sa severa u odnosu na žene. Tok bolesti je uticao na nivo 25(OH)D u serumu. Nivo 25(OH)D bio je u značajnoj korelaciji sa kliničkom nesposobnošću (Spearmanov r = -0.23, p = 0.001) i progresijom bolesti (Spearmanov r =

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-0.25, p = 0.0004). **Zaključak.** Serumske koncentracije 25(OH)D povezane su sa nesposobnošću i napredovanjem bolesti kod bolesnika sa MS. Niži nivoi 25(OH)D nađeni su kod žena sa severa. Nađene niske koncentracije 25(OH)D ne mogu se objasniti samo geografskom širinom i nedovoljnim izlaganjem suncu, zbog čega su potrebna dalja genetska istraživanja.

Ključne reči:
multipla skleroza; vitamin d; geografija, medicinska; bolest, indeks težine; bolest, progresija; pol, faktor; crna gora.

Introduction

Multiple sclerosis (MS) is a complex neurological disorder which etiology is still unknown. Researchers in this area believe that the interaction of several different factors (genetic predisposition and environmental factors) may be involved. Although heritage plays an important role in the pathogenesis, migration and other studies have shown that the environment is very important for its development.

Epidemiological studies suggest that living in certain geographical areas and/or migration in these areas before the age of 15 increases the incidence of MS. The incidence of MS is low in the tropics and increases with distance from the equator towards both hemispheres [1, 2]. Thus, under-exposure to UV rays can predispose a person to develop MS later on [3]. This hypothesis has led to an extensive research of potential role of vitamin D in MS. The possible relationship is further confirmed by the observation of a lower risk of developing MS in areas with high consumption of fatty fish rich in vitamin D, despite the unfavorable latitude [4]. Over the last 50 years there has been a dramatic increase in the incidence of not only MS but also other autoimmune and immune-mediated diseases. This increase is presumably caused by the changes in our environment, rather than genetic changes, which would take much longer to manifest themselves [5]. Dobson et al. [6] in their study indicated more clinical relapses and MRI activities in the spring/summer and less in the autumn/winter season in the northern hemisphere [6]. The reverse case was observed in the southern hemisphere [7].

In addition to its fundamental role in the homeostasis of calcium and bone metabolism, there is growing evidence that vitamin D has additional, immunoregulatory function, making it a promising candidate in the pathogenesis and treatment of autoimmune diseases like MS [8].

In patients already diagnosed with MS, a number of studies suggested that the consistently higher serum vitamin D levels were associated with the favorable course of the disease [9–11]. In a small Finnish study, lower concentration of 25-hydroxyvitamin D [25(OH)D] were measured in patients having their first relapse compared to healthy individuals in the control group [9]. The concentration of 25(OH)D were significantly lower during relapse compared to periods of remission, which could indicate the regulatory role of vitamin D in the disease activity [12].

The aim of this study was to assess the possible difference between 25(OH)D serum levels in MS patients from north and south of Montenegro and to investigate the association between 25(OH)D serum levels and disability in MS patients.

Methods

A total of 196 patients, aged 18 to 65 years, with clinically and magnetic resonance imaging (MRI) confirmed MS, using McDonald criteria [13] were recruited from consecutive patients admitted to the Neurology Outpatient Clinic in the period 2013–2015. Patients were coming from the southern, northern and central areas of the country. Twenty-four patients from the central area were grouped together with the patients from the North due to very similar amount of registered sunny days in both parts of the country. Written informed consent was obtained from all study participants, and the study was approved by the local ethics committee.

Blood for the analysis was taken from each patient on the day of admission (during late spring and summer – late April till the end of August 2013, 2014 and 2015). The blood samples were taken from patients exclusively during remission. Samples were shielded from direct light after collection and stored at -20°C. Chemiluminescence immunoassay (COBAS-e601, Rosche) was used to determine the 25(OH)D levels. According to Smolders et al. [12] it was proposed that a 25(OH)D concentration of 70–80 nmol/L is adequate for a normal calcium metabolism [12]. The patients recruited for the study were not on any kind of vitamin D supplementation prior to blood sampling.

The control group consisted of 116 subjects from the North and 80 subjects from the South of Montenegro. They were collected among random sample of medical staff employed in health centers from three cities in North (Berane, Bijelo Polje and Pljevlja) and from Clinical Centre of Montenegro (Podgorica) from the South. They had no prior or current history of any known disease (on the sampling day) and they were age matched to MS patients.

Demographic characteristics of MS patients and clinical variables regarding MS phenotype were retrieved from our patient database and included sex, age, date of birth, residence as well as relapse remitting (RR) MS, secondary progressive (SP) MS and primary progressive (PP) type of MS. Patients with PP MS were excluded from further analysis due to a very small number of patients (only two). Database also included age of disease onset, imaging results (brain and spine MRI), spinal tap results and isoelectric focusing of cerebrospinal fluid and serum. Expanded Disability Status Scale (EDSS) was scored on the day of admission prior to blood sampling [14]. Disease severity was estimated using the Multiple Sclerosis Severity Score (MSSS) [15] which corrected the EDSS for disease duration at the moment when blood samples were taken.

Official data regarding the number of sunny days for the cities from the North, South and Central parts of Monte-

negro were obtained from the Institute of Hydrometeorology and Seismology of Montenegro [16]. Data included the average amount of sunshine during the period from 1976–1985, when our participants were born, and during 2013–2015 when study was being conducted.

Statistical analysis

Statistical software (Stat Soft Inc, version 8) was used for statistical analysis. Mean values are provided with standard deviation (± SD) and mean differences (MD) with standard error (± SD). Normality test was performed using Kolmogorov-Smirnov test with Lillieform's correction for all continuous variables. As the distribution of investigated variables was skewed, the Mann-Whitney *U* test was used for the comparison of differences between two independent variables. The Spearman's rank correlation test was used to investigate the correlation of given variables. A 2-sided *p* value < 0.05 was considered statistically significant.

Results

Description of the study population is presented in Table 1. The 137 patients with RR and 59 patients with SP form of MS were included in the study. One hundred and sixteen patients (80 females and 36 males) were from the North and 80 (62 females and 18 males) from the South of Montenegro.

According to the official data from the Institute of Hydrometeorology and Seismology of Montenegro, there were two time periods (1976–1985 and 2013–2015) when the average number of the sunny days in the north was significantly lower than in the south (53.2 ± 22.9 *vs* 106.5 ± 9.8, Mann-Whitney *U* test $p < 0.01$ and 56.4 ± 24.9 *vs* 109.2 ± 24.4, Mann-Whitney *U* test $p < 0.01$, respectively). The measurements of sunny days were conducted in 5 cities in the south and 9 cities in the north. There was no significant difference in the number of sunny days between the two periods of time (1976–1985 vs 2013–2015) neither in the south (106.5 ± 9.8 vs 109.2 ± 24.4, *p* non significant) nor in the north (53.2 ± 22.9 *vs* 56.4 ± 24.9, *p* non significant).

The serum levels of 25 (OH) D were significantly lower in MS patients compared to controls ($p < 0.001$) as shown in Table 2. The controls (141 female and 55 male, average age: 42.3 ± 11.6) were matched to the patients according to the age. Vitamin D significantly correlated with age in controls ($p = 0.004$), but not in MS patients ($p = 0.30$). The serum 25(OH)D was significantly different in regard to gender with women showing lower levels in both, MS patients and controls (Table 2).

Table 1

Clinicodemographic characteristics of the multiple sclerosis (MS) patients

Parameter	Patients		
	MS total, (n = 196)	RR MS, (n = 137)	SP MS, (n = 59)
Age (years), mean ± SD	41.2 ± 10.9	38.0 ± 9.7	48.5 ± 9.9
Gender (female/male), n	142/54	98/38	44/16
Disease onset age (years) , mean ± SD	31.3 ± 9.8	30.6 ± 9.5	32.7 ± 10.5
Disease duration (years) , mean ± SD	12.2 ± 16.2	10.0 ± 17.0	17.3 ± 13.2
EDSS, mean ± SD	3.7 ± 1.9	2.8 ± 1.2	5.5 ± 1.7
MSSS, mean ± SD	5.2 ± 2.3	4.7 ± 2.2	6.1 ± 2.1

Values of continual parameters are presented as mean ± standard deviation (SD); n – number of subjects; EDSS – expanded disability status scale; MSSS – MS severity score; RR MS – relapse remitting MS; SP MS – secondary progressive MS.

Table 2

Serum levels of 25-hydroxyvitamin D [25(OH)D] in the study participants

Subjects	n	Vitamin D (nmol/L)	*p* (M-W U Test)
Controls, total	196	61.24 ± 24.94	$< 10^{-6}$***
Patients, total	196	41.80 ± 27.51	
Controls gender			
female	141	57.23 ± 19.62	0.0002**
male	55	71.72 ± 33.24	
Patients gender			
female	142	38.61 ± 26.24	0.01 *
male	54	49.83 ± 29.74	
Controls origin			
North Montenegro	116	63.93 ± 27.15	0.07
South Montenegro	80	57.29 ± 20.82	
Patients origin			
North Montenegro	116	44.07± 30.16	0.40
South Montenegro	80	38.76 ± 23.12	
Patients, type of MS			
RR	137	45.11 ± 28.95	0.007**
SP	59	34.12 ± 22.23	

Vitamin D levels are presented as mean ± standard deviation (SD); MS – multiple sclerosis; n – number of subjects; RR MS – relapsing-remitting MS; SP MS – secondary progressive MS; M-W *U* test – Mann-Whitney *U* test; * – statistical significance when $p < 0.05$;

**** – statistical significance when $p < 0.01$; *** – statistical significance when $p < 0.001$.**

The significantly lower vitamin D serum levels were registered in patients with SP MS, compared to RR MS (Table 2).

Although the entire group of patients had no statistically significant correlation among the 25(OH)D levels and their residence (divided by latitude 42′38″ north and south), the significantly higher levels of 25(OH)D were detected in men from the north compared to women (Figure 1a). In the south, the 25(OH)D levels were similar in both men and women (Figure 1b). The same relation was found between levels of 25(OH)D and residence, by latitude, in controls. Only in the north, men (n = 36) had significantly higher 25(OH)D compared to women (n = 80) (77.27 ± 33.93 mmol/L, *vs* 57.93 ± 21.10 mmol/L, respectively, M-W *U* test $p = 0.0006$).

Vitamin D levels also significantly correlated with clinical parameters of both, disability (EDSS, Spearman's r = -0.23, $p = 0.001$) and progression (MSSS, Spearman's r = -0.25, $p = 0.0004$) of MS in entire patient group. The significant correlation with MSSS was found in the north (MSSS, Spearman's r = -0.22, $p = 0.02$) and the south groups (MSSS, Spearman's r = -0.25, $p = 0.03$) and in women (MSSS, Spearman's r = -0.32, $p = 0.0002$), but not in men (MSSS, Spearman's r = -0.14, $p = 0.3$).

Discussion

In this study significantly lower serum levels of 25(OH)D were detected in MS patients compared to controls. Similar results were obtained in a study by Soilu-Hänninen et al. [17] who compared the levels of vitamin D in patients with MS with healthy controls. They found the level of vitamin D to be significantly lower during the summer among MS patients compared to controls, while the difference between the groups was not found during the winter period. Other studies that investigated the correlation between the MS phenotype and levels of vitamin 25(OH)D reported that 25(OH)D serum levels were significantly lower in the RR patients compared to the healthy controls [18, 19].

We found that women both in patient and in control group had significantly lower 25 (OH) D serum levels than men. Kragt et al. [9] concluded that higher levels of vitamin D in women reduced the risk of MS, while the lower level was negatively correlated with EDSS findings in the study group.

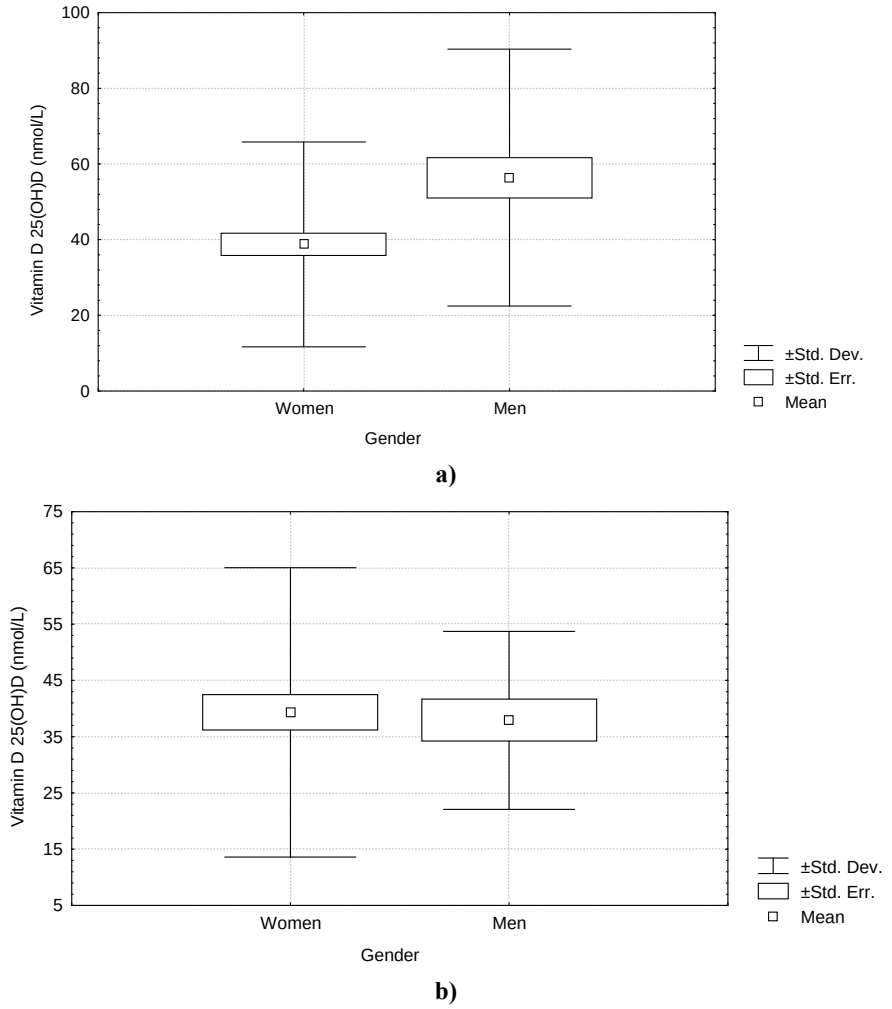

Fig. 1 – The serum concentration of 25-hydroxyvitamin D [25-(OH)D] in: a) women (n = 80) and men (n = 36) from the north (M-W *U* test, p = 0.004); b) women (n = 62) and men (n = 18) from the south (M-W *U* test *p* = 0.62). M-W *U* test – Mann-Whitney *U* test.

The authors implied clues to the pathogenesis of the gender difference in risk and to the nature of the environmental factors involved in MS. Authors of this manuscript cannot offer proper explanation for the lower levels of vitamin D among women in the control group. Possible reasons, besides latitude (above 40th parallel), include several factors such as low sun exposure, genetic factors, a large proportion of smokers in the population of Montenegro and nutrition. We also found significantly lower 25(OH)D serum levels among the patients with SP MS compared to the RR patients. Comparable results were found in a Dutch study, in which both metabolite levels were significantly lower in the progressive forms compared to the relapsing remitting MS phenotype [12]. Serum levels of 25(OH)D in our study group were associated with both disability and progression of MS. Smolders et al. [19] noted a similar effect of low levels of vitamin D in course of the disease, i.e. a lower level of of 25(OH)D during relapses compared to the periods of remission. These results are suggestive for a disease modulating effect of the serum concentrations of 25(OH)D on MS.

The effect of latitude on the risk of MS has long been known and it is universally acknowledged; prevalence of the disease being minimal at the equator and increasing with either north or south latitude [20]. Accordingly, a problem of vitamin D supply affects a larga number of people, namely those who live beyond the 40th parallels North or South [21, 22]. Relatively limited amounts of sun are mainly related to Canada, the northern part of the United States, almost all of Europe (40th parallel runs through the center of Spain), Russia and several areas in the southern hemisphere, such as New Zealand, Tasmania and Patagonia which include only about 15% of the world's population while the remaining 85% live in sunny regions [22].

Montenegro extends between 41º51' and 43º33' north latitude, which according to the above mentioned data suggests that lower levels of vitamin D should be expected in our country as well, in both general population and among MS patients. In our study group, serum levels of vitamin D were significantly lower in MS patients compared to controls, which is in accordance with other similar studies [23]. Further, health controls had a low mean level of vitamin 25 (OH) D. Recent epidemiological studies conducted in 40 countries, located mainly above 40th parallel, showed low levels of 25(OH)D among adult Caucasians [24]. Zadshir et al. [25] found mean serum level of 25(OH)D to be 74 nmol/L in a large cohort distributed throughout the US. However, a more recent analogous American cohort revealed even lower mean serum vitamin D level. Low mean serum 25(OH)D levels were also reported among healthy adult population in Australia [26], New Zealand [27] and Canada [28].

In our study the serum levels of vitamin D in patients from both groups (north and south) were low. The significantly higher levels of vitamin D were registered in male patients from the north compared to the female ones. In the south, the vitamin D levels were similar in both genders**.** Higher serum levels of vitamin D in men from the north could be explained by cultural milieu in Montenegro (the north of Montenegro is rural and men care about household and therefore are more exposed to the sun. Women are mainly involved in household chores. Likewise, the great proportion of inhabitants from the northern parts of a country are Muslims and the women wear traditional skin covering clothes. Other authors presented different results in regard to gender. Women tended to have borderline significantly higher mean serum level of 25(OH)D than men [29].

Our study did not detect the difference in vitamin D levels among MS patients compared to latitude which is quite the opposite what other studies have reported. Even though there was found significantly lower average number of sunny days in the north than in the south, the result we obtained can be partly explained by a small difference in latitudes between this two regions. On a world-wide scale, in a meta-analysis based on 394 studies, a significant correlation was observed between 25(OH)D serum levels and latitude in Caucasian subjects [28]. In Nordic countries the serum levels of vitamin D are often lower compared to countries with more sunny days [29], whereas in tropical regions the serum levels are generally higher [30].

However, exceptions are not infrequent. The reason may be due to differences in lifestyle, cultural habits or diet. Low serum levels of vitamin D can be found in people from sunny countries, if they avoid the sun, or, on the other hand, relatively high serum levels in people of Northern regions, who take more advantage of the sun in summer and consume diet rich in vitamin D in winter [31].

Conclusion

The serum levels of vitamin D were lower than expected in the entire study group. The serum levels of 25(OH)D were significantly lower in the MS patients and were associated with disability and progression. There was no significant difference in the levels of vitamin D among patients from the north and south of Montenegro, although the amount of sunshine in the south is significantly higher. The male patients from the northern part of the country had significantly higher level of vitamin D than women. The low levels of vitamin D cannot be explained solely with unfavorable latitude and insufficient sun exposure, thus further genetic analysis is needed.

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