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Vitamin D status in patients with multiple sclerosis: an association with insolation, disease course, and HLA-DRB1 gene polymorphism

Recent systematic reviews and a meta-analysis have shown that there is insufficient evidence on the relationship of multiple sclerosis (MS) to vitamin D supplementation

Objective: *to assess the relationship of vitamin D status in MS patients to insolation, disease course, and HLA-DRB1 gene polymorphism.*

Patients and methods. *The one-stage study enrolled 90 patients with relapsing-remitting MS (a study group) and 87 volunteers without this disease (a control group). The enrolled were born and live in the Altai Territory of the Russian Federation. The serum level of 25(OH)D was measured by enzyme immunoassay.*

Results and discussion. *25(OH)D <30 ng/ml was more common in patients with MS than that in the controls. There were no intergroup differences in the time spent in the sun for 6 months before inclusion in the study (p=0.020). The level of 25(OH)D was higher in the high insolation period from April to September than that in the low insolation period from October to March in both patients with MS (p<0.005) and controls (p<0.001). There was no association of 25(OH)D levels with urban and rural residence, gender, age at MS onset, severity of neurological disorders, their progression rate, and MS risk alleles within the HLA-DRB1 gene (03, 13, 15).*

Conclusion. *Vitamin D deficiency is more common in patients with MS than in those without this disease. This is unlikely to be due to the differences in the radiation received from the sun. The final conclusion on the relationship of vitamin D to MS can be made after obtaining the results of a prospective follow-up.*

Keywords: *multiple sclerosis; vitamin D; insolation; HLA-DRB1.*
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For reference: *Smagina IV, Lunev KV, Elchaninova SA. Vitamin D status in patients with multiple sclerosis: an association with insolation, disease course, and HLA-DRB1 gene polymorphism. Nevrologiya, neiropsikhiatriya, psikhosomatika = Neurology, Neuropsychiatry, Psychosomatics. 2020;12(3):63–68.*
DOI: *10.14412/2074-2711-2020-3-63-68*

Multiple sclerosis (MS) is a chronic multifactorial CNS disease characterized by autoimmune-inflammatory and neurodegenerative lesions with polymorphic clinical manifestations [1]. Genome-wide studies have shown that genetic features predisposing to MS are formed by hundreds of independent and interacting polymorphic genes, most of which are associated with the immune system [2]. Carrying certain alleles of the *HLA-DRB1* gene, which encodes the beta chain of the class II histocompatibility antigen on antigen-presenting cells, affects the phenotypic variability of MS risk most strongly (up to 10.5%) [2].

Rare exposure to the sun, low insolation of the residential area, air pollution from industrial enterprises and automobile transport, certain viral infections, obesity and other factors are considered external risk factors for MS [1]. The relationship between MS and sufficiency of vitamin D has been intensively studied in recent years. The main source of this vitamin for humans is the synthesis of vitamin D_3 in the skin from 7-dehydrocholesterol under the influence of the ultraviolet part of the solar radiation spectrum [3]. The biologically active form of vitamin D resulting from its metabolism is the steroid hormone calcitriol. It is assumed that the insufficiency of this hormone in combination with pleiotropic effects, including not only the regulation of calcium homeostasis, but also immunomodulation, can affect the initiation and persistence of immune inflammation and neurodegeneration in the CNS in MS [4]. One of the implementing mechanisms of this effect may involve the regulation of vitamin D-sensitive genes of the immune system, in particular the *HLA-DRB1* gene [5].

This hypothesis is based on the data from several prospective studies indicating a decrease in the risk of MS as vitamin D intake increases [6], as well as reports of the association of vitamin D deficiency in MS patients with the frequency of the disease exacerbations, the appearance of new demyelination foci and an increase in the volume of brain damage according to magnetic resonance imaging [7–9]. It was found in a number of populations that the plasma level of the vitamin D metabolite used as an indicator of vitamin D sufficiency, 25-hydroxy-vitamin D (25(OH)D) [3], is significantly lower in MS patients compared with volunteers without this disease [10]. However, the discussion about the causes of this phenomenon and its relationship with reduced insolation, which is associated with a well-known increase in the prevalence of MS, is continuing [4].

Recent systematic reviews and a meta-analysis have shown that there is insufficient data to prove the relationship

between MS and vitamin D supplementation [11, 12], which indicates the need for additional controlled studies in this field. Taking into account the multifactorial nature of MS, differences in the population's gene pool, climatic and environmental conditions of residence places, it is relevant to study the relationship between the status of vitamin D and MS in different regions of Russia.

Objective: to assess the relationship between vitamin D status in MS patients and insolation, the course of the disease, and HLA-DRB1 gene polymorphism.

Patients and methods

Ninety patients with remittent MS in remission (the main group) and 87 volunteers without MS (the control group) took part in the study.

The criteria for non-inclusion in the study were autoimmune diseases other than MS, chronic diseases of the gastrointestinal tract, kidneys, bariatric interventions, taking medications or food supplements with vitamin D and/or its active metabolites for 3 months before inclusion in the study. Additional criteria for non-inclusion were the MS onset before the age of 18, neurological disorders of more than 6 points on the Expanded Disability Status Scale (EDSS), and exacerbation of MS less than 6 months ago.

All study participants were born and permanently resided in the Altai region of the Russian Federation, and were Caucasian by phenotypic characteristics. Most of MS patients received disease-modifying treatments (DMT). The characteristics of the main and control groups are presented in Table 1.

The McDonald criteria 2010 [13] were used for the diagnosis of MS, and the Kurtzke system (EDSS) was used for assessing the disease severity and disability [14]. The rate of progression (RP) of MS was calculated by dividing EDSS scores by the duration of the disease in years at the time of examination. Slow RP MS (⩽0.25 points/year) was found in 41 (45.5%) patients, medium (0.25<SP⩽0.75 points/year) – in 33 (36.7%) patients and high (>0.75 points/year) – in 16 (17.8%).

Obesity was determined by body mass index (BMI) in accordance with WHO criteria [15].

The vitamin D status was evaluated in accordance with the recommendations of the Russian Association of Endocrinologists [16] for the concentration of 25(OH)D, which was measured in the venous blood serum by enzyme immunoassay using Euroimmun AG (Germany) reagents. Insolation data from the website of the National Aeronautics and Space Administration of the United States *(https://eosweb.larc.nasa.gov)* were used.

Molecular genetic studies were performed with the participation of M.L. Filippenko, a specialist of the Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk). DNA isolation was performed by a standard procedure, including separation and lysis of blood cells, followed by proteinase K protein hydrolysis, and purification of DNA with phenol-chloroform and ethanol precipitation. Genotyping was performed using TaqMan probes on the Icycler iQ5 amplifier (Bio-Rad, USA).

The program Statistica 13.0 (Stat. Soft Inc., USA) was used for statistical analysis. Differences in categorical variables were evaluated by the Fischer criterion. Differences in the quantitative variables of two independent groups were evaluated by the Mann-Whitney U-criterion, three or more independent groups – by the Kraskel-Wallis H-criterion, and relationships between variables – by the Spearman correlation coefficient (rs) and the Odds Ratio (OR). The accordance of the *HLA-DRB1* genotype distribution to the Hardy-Weinberg equilibrium was validated using the χ^2 criterion in the Definetti program on the website of the Institute of Human Genetics (Munich, Germany; *https://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl*). This correlation was found for both the control group (p=0.375) and the main group (p=0.276). Quantitative variables are represented as the Mean (M) and Standard Deviation (±SD), and in some cases as the 95% Confidence Interval (CI).

Table 1. *Characteristics of the main and control groups*

Characteristic	Main group (n=90)	Control group (n=87)	P-value
Age, years, M±SD Посчитано	34.8±8.4	34.6±12.2	0.269
Men : women	36:54	41:46	0.364
Disease onset age, years, M±SD	28.3±8.0		
Duration of the disease, years, M±SD	6.8ё6.4		
EDSS, points, M±SD	1.9±1.3		
Disease progression rate, points/year, M±SD	0.46±0.37		
Frequency of exacerbations of MS, number of exacerbations/year, M±SD	0.68±0.50		
Patients receiving DTM, n (%), including:	60 (66.7)		
teriflunomide	2 (2.2)		
interferon beta-1b	7 (7.8)		
glatiramer acetate	24 (26.7)		
interferon beta-1a	27 (30.0)		
Patients receiving non-DTM, n (%)	30 (33.3)		

Results

It was found that 25(OH)D level in the blood serum of MS patients was lower compared with the control group (Table 2).

Vitamin D deficiency and insufficiency were registered in some of the participants in both groups (Table 3). However, an inadequate 25(OH)D level corresponding to vitamin D deficiency or insufficiency occurred in MS patients about 2 times more often (in 72.2%) than in the control group (in 36.8%). It was found by logistic regression analysis that MS is associated with inadequate vitamin D status (OR 4.31; 95% CI 2.29–8.12; p<0.001).

The level of 25(OH)D in blood serum was higher in both men and women of the control group than in similar subgroups of MS patients. No differences in 25(OH)D level were found in any of the groups between men and women (see Table 2). The status of vitamin D in rural and urban residents was similar both in the control group and in the group of MS patients (Table 4).

Taking into account the geographical location of the Altai region, the average monthly values of total solar radiation were calculated and two calendar periods were allocated – with high (from April to September) and low (from October to March) insolation (Table 5).

The concentration of 25(OH)D in the blood serum during the period of high insolation was higher than during the period of low insolation, both in MS patients and in the control group, while maintaining intergroup differences (see Table 5). According to a survey of study participants, there were no differences between the groups in the duration of exposure to solar radiation for 6 months before study (2.6±1.7 and 2.9±2.0 hours/day in the main and control groups, respectively; p=0.020).

The total solar radiation received during intrauterine development was less in those born from April to November compared with those born from December to March (24.9; 95% CI 21.6–29.5 and 35.2; 95% CI 33.0–36.1 kW*h/m^2 per day, respectively; p=0.008). However, there were no differences in this indicator between MS patients and the controls (p=0.535).

The BMI above the normal limit (18.5–24.9 kg/m^2) was revealed only in the men of the control group (Table 6). This value corresponded to overweight (BMI 25.0–29.9 kg/m^2) and positively correlated with BMI (rs=0.33; p=0.037) in this subgroup only. This relationship was not found in women of the control group, as well as in men and women with MS (rs=-0.074; p=0.633; rs=0.09; p=0.610; rs=-0.20; p=0.140, respectively).

Correlations of 25(OH)D level with the age of onset, the duration of the first MS remission, the severity of neurological disorders at the time of examination, and the retrospectively evaluated RP of MS were not detected in MS patients (Table 7).

The differences in 25(OH)D levels between subgroups of MS patients treated with different DTM and without DTM were not detected (H=1.71; p=0.888).

A previous study in the Altai region found that MS risk alleles are *HLA-DRB1*3, HLA-DRB1*13*, and *HLA-DRB1*15* [17]. There was no difference in 25(OH)D level in the carriers of these alleles and carriers of other alleles of the *HLA-DRB1* gene (Table 8).

Discussion

The wide prevalence of inadequate vitamin D status in MS patients compared with those without MS in the Altai region is consistent with the results of examination of MS patients in other regions [4, 10].

Table 2. *The blood serum concentration of 25(OH)D in the main and control groups, ng/ml*

Group	Main group	Control group	P-value
Whole group, *1*	26.1±6.9 (n=90)	33.4±8.9 (n=87)	<0.001
Men, *2*	25.1±6.5 (n=36)	34.2±8.6 (n=41)	<0.001
Women, *3*	26.8±7.2 (n=54) p_{2-3}=0.367	32.6±9.2 (n=46) p_{2-3}=0.361	0.001

Note: The significance of the differences between the indicator values in the main and control groups is presented here and in Tables 4–6.

Table 3. *Distribution of the vitamin D status among individuals of the main and control groups*

Vitamin D status	25(OH)D, ng/ml	Main group (n=90), n (%)	Control group (n=87), n (%)	P-value
Severe deficit	<10	0	0	NI
Deficit	10–20	17 (18.9)	7 (8.0)	0.029
Insufficiency	20–30	48 (53.3)	25 (28.8)	<0.001
Adequate level	>30	25 (27.8)	55 (63.2)	<0.001

Note: NI – not identified due to lack of appropriate status among study participants.

Table 4. *Concentration of 25(OH)D in blood serum in the main and control groups depending on the place of residence*

Place of residence	Main group	Control group	P-value
City, *1*	26.6±6.3 (n=57)	32.9±9.2 (n=74)	<0.001
Village, *2*	25.4±8.0 n=33 p_{1-2}=0.151	36.3±6.3 (n=13) p_{1-2}=0.234	<0.001

Table 5. *The concentration of 25(OH)D in the blood serum in the main and control groups during periods with different levels of solar radiation*

Period of the year	Total solar radiation, kW o h/m^2 per day	25(OH)D, ng/ml Main group	25(OH)D, ng/ml Control group	P-value
April – September, *1*	4.89±1.02	28.4±5.9 (n=39)	39.6±4.0 (n=27)	<0.001
October – March, *2*	1.54±0.89 p_{1-2}=0.002	24.4±7.2 (n=51) p_{1-2}=0.005	30.6±9.1 (n=60) p_{1-2}<0.001	<0.001

The absence of differences in the vitamin D status between men and women in both the control group and the main group suggests that the prevalence of MS in women in comparison with men, characteristic of all populations studied to date, including the population of the Altai region [1, 18], is not associated with differences in the vitamin D status. The relationship of 25(OH)D with another feature of MS epidemiology in the Altai region – a greater prevalence of MS in cities compared with villages [18], possibly due to urban air pollution from industrial and transport emissions, was not found.

Higher 25(OH)D levels in both MS patients and in the control group during the period of the year with high insolation compared to the period with low insolation, obviously reflect the stimulating effect of solar radiation on the biosynthesis of vitamin D3 in the skin. At the same time, these results, in our opinion, indicate a low probability that the level of solar radiation and its seasonal variation in the Altai region are factors that determine the wider prevalence of vitamin D deficiency in MS patients compared with the controls. This is indicated by reduced levels of 25(OH)D in MS patients compared with the control group in each of the insolation periods.

Some publications have reported that MS is associated with total solar radiation received during prenatal development, which is hypothetically explained by the indirect influence of insolation on the formation of immunoreactivity features that appear after birth [19]. However, according to a systematic review and meta-analysis, there is insufficient evidence of this relationship [19]. In our study in the Altai region, no such association was found.

In accordance with the comparability of BMI in MS patients and in the control group, the high prevalence of inadequate vitamin D status in MS patients cannot be explained by the influence of adipose tissue. This does not contradict the data on the association of adipose tissue mass with vitamin D status [20], since increased BMI (overweight) was detected in men of the control group only and positively correlated with 25(OH)D level. It is possible that the relationship of vitamin D metabolism with adipose tissue is manifested in the presence of excessive accumulation of this tissue only. We cannot exclude divergent effects of adipose tissue on 25(OH)D levels in patients with different degrees of obesity.

The absence of a relationship between the 25(OH)D level and such clinical features of the course of MS as the age of the onset of the disease, the duration of the first remission, the severity of neurological disorders, and the rate of their progression is consistent with data from other authors [21]. In general, the results of the observations done so far do not allow to make an unequivocal conclusion on the association of clinical characteristics of MS with vitamin D status [4, 12].

The absence of specific features of the vitamin D status in the carriers of the *HLA-DRB1* MS risk alleles in comparison with the carriers of other alleles of this gene may indicate the independence of the influence of *HLA-DRB1* gene polymorphism and vitamin D deficiency, if any, on the risk of MS. This assumption was made by other researchers [22], and, in our opinion, it does not contradict the data on the immunomodulating action of calcitriol in MS through vitamin D-sensitive elements in the promoter region of the *HLA-DRB1* gene [23]. It is possible that this mechanism may contribute to the favorable course of the disease in the treatment of patients with vitamin D preparations and its bioactive metabolites.

It should be noted that the presented results of vitamin D status of in MS patients with the disease onset after 18 years are similar to the previous data for pediatric MS in the Altai region [24].

Table 6. *BMI in the main and control groups, kg/m²*

Group	Main group	Control group	P-value
Whole group, *1*	23.8±5.8 (n=90)	24.3±5.0 (n=87)	0.361
Men, *2*	24.6±8.3 (n=36)	26.6±4.2 (n=41)	0.002
Women, *3*	23.2±3.4 (n=54) p_{2-3}=0.618	22.2±4.7 (n=46) p_{2-3}<0.001	0.040

Table 7. *Correlation of 25(OH)D level with clinical characteristics of MS course*

Clinical characteristics	Spearman's correlation coefficient	P-value
Age of disease onset, years	-0.07	0.497
Duration of the first remission, months	0.11	0.368
Duration of the disease, years	0.14	0.180
Severity of neurological disorders, EDSS, points	0.13	0.226
Disease progression rate, points/year	-0.04	0.725
Frequency of exacerbations of MS, number of exacerbations/year	0.02	0.860

Table 8. *Concentration of 25(OH)D in the blood serum of patients with MS-carriers of HLA-DRB1 alleles associated with MS risk*

HLA-DRB1 allele	MS patients, n (%)	25(OH)D, ng/ml	Differences between allele carriers	Differences compared to carriers of other alleles HLA-DRB1, P-value
03	14 (15.6)	27.6±7.9	H=0.48; p=0.781	0.531
13	5 (5.6)	28.4±5.0		0.384
15	46 (51.1)	27.1±6.6		0.460

Conclusion

The results of the study indicate that in the Altai region, vitamin D insufficiency/deficit in patients having remittent MS with the onset after 18 years of age, is more common than in individuals without MS. This cannot be explained only by the dependence of vitamin D status on seasonally varying insolation and/or the duration of exposure and area of the body surface exposed to solar radiation. It is unlikely that the vitamin D status depends on gender and fat mass in the absence of obesity in both MS patients and residents of the region without MS. The association of vitamin D deficiency with the features of the course of MS that debuted in adulthood, as well as with the carriage of the alleles of MS risk of the *HLA-DRB1* gene, including *HLA-DRB1*15*, is also unlikely. The final conclusion about the relationship of the dynamics of increasing neurological deficit in MS and the status of vitamin D can be made based on the results of prospective observations.

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Received/Reviewed/Accepted
22.03.2020/30.04.2020/10.05.2020

Conflict of Interest Statement

The investigation has not been sponsored. There are no conflicts of interest. The authors are solely responsible for submitting the final version of the manuscript for publication. All the authors have participated in developing the concept of the article and in writing the manuscript. The final version of the manuscript has been approved by all the authors.

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