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Original Scientific Paper

RETROSPECTIVE OBSERVATIONAL STUDY OF VITAMIN D STATUS IN NEUROLOGICAL PATIENTS

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SUMMARY – The main objective of this study was to investigate the prevalence of insufficient serum 25-hydroxyvitamin D level in a sample of neurological patients and to compare it to the estimate in the general Croatian population. The secondary aim was to test for the possible significant differences in vitamin D2 insufficiency between the groups of patients according to gender, season, region, vitamin D3 supplementation, and diagnosis of autoimmune disease. This retrospective study involved 371 neurological patients in one-year period. The data collected included gender, age at the time of admission, region, season at the time of admission, main neurological diagnosis at discharge, serum 25-hydroxyvitamin D level at the time of hospitalization, and use of vitamin D3 substitutes prior to hospitalization. The proportion of neurological patients with 25-hydroxyvitamin D insufficiency (<75 nmol/L) was estimated at 74.66% (95% confidence interval, p<0.0001), which is slightly lower than in the Croatian general population. There were no significant differences in 25-hydroxyvitamin D insufficiency according to gender, season and region. Significant differences in 25-hydroxyvitamin D insufficiency were found according to vitamin D3 supplementation and diagnosis of autoimmune disease.

Key words: *Vitamin D deficiency, Multiple sclerosis; Autoimmune diseases*

Introduction

Vitamin D is a fat-soluble secosteroid that exists in two main forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol)[1]. Its active metabolite calcitriol plays a role not only in skeletal health and disease but also in immune reactions, autoimmune diseases, glucose absorption control, antihypertensive reaction, wound repair, neuroprotection, and antiproliferative actions[2,3]. Calcitriol mechanism of action comprises of non-genomic mechanism and genomic mechanisms *via* nuclear vitamin D receptor (VDR)/retinoid X receptor (RXR) complex and effect on activated T and B lymphocytes, neutrophils, macrophages, and dendritic cells[4]. Serum calcidiol level is a marker of vitamin D status, as it is substrate-dependent[5]. Currently, there is no consensus on adequate vitamin D intake and optimal serum calcidiol level regarding all potential vitamin D effects[6]. Different experts from different points of view have proposed optimal serum calcidiol levels, ranging from 20 ng/mL (~50 nmol/L)[7] or at least 30 ng/mL (~75 nmol/L)[5]. Serum calcidiol levels less than 50 nmol/L indicate a deficiency, levels between 52 and 72 nmol/L indicate insufficiency, and levels equal or more than 75 nmol/L usually indicate sufficiency[8], whereas levels equal or higher than 374 nmol/L indicate toxicity[9]. Surveys from the past decade have shown that vitamin D deficiency and insufficiency became very common in general population

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of most countries around the world, representing a global problem of public health[10]. Evidence in favor of lifelong vitamin D effects on the nervous system, especially central nervous system, suggests a possible association of vitamin D deficiency/insufficiency and neurological disorders[11,12]. Considering the important role of vitamin D in neurological diseases with the possibility of therapeutic influence on its level, but currently insufficient data on the status of vitamin D in the population of neurological patients in Croatia, there is a need to get an insight into the vitamin D status and risk factors for vitamin D deficiency/insufficiency in the group of neurological patients.

The main aim of this study was to estimate the prevalence of insufficient serum 25-hydroxyvitamin D [25(OH)D] level (<75 nmol/L) in a sample of neurological patients and to compare it to the estimate in the general Croatian population[13]. The secondary aim was to test for the possible significant differences in vitamin D2 insufficiency between groups of patients according to the potential key predictors of vitamin D2 insufficiency (such as gender, season, region, vitamin D3 supplementation, and diagnosis of autoimmune disease).

Patients and Methods

The study was conducted as a retrospective analysis on 371 patients aged ≥18 with determined serum 25(OH)D level and hospitalized at the Division of Neuroimmunology, Neurogenetics and Pain, Department of Neurology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia, during a one-year period, from March 2021 until March 2022.

Data were collected from the electronic data base of the Sestre milosrdnice University Hospital Center (hospital information system) and Department manual register of patient admission and discharge data kept by the Department Head Nurse. The data collected included gender, age at the time of admission, region (inland or littoral Croatia), season at the time of admission (one subgroup from March, 8, 2021 till September, 31, 2021 and the other subgroup from October, 1, 2021 till March, 8, 2022), main neurological diagnosis at discharge (additionally categorized into neurological autoimmune or neurological non-autoimmune disease), serum 25(OH)D levels at the time of hospitalization, and use of vitamin D3 substitutes prior to hospitalization. The level of 25(OH)D was determined at the Hospital Biochemical Laboratory using chemiluminescent microparticle immunoassay (CMIA) on Architect i2000 analyzer (Abbott, Abbott Park, USA). Serum 25(OH)D level was expressed in nmol/L and categorized according to the reference values of the Hospital Biochemical Laboratory, as follows: 25(OH)D deficiency (<25 nmol/L) and 25(OH)D insufficiency (i.e., inadequate 25(OH) D level (<75 nmol/L)). As the focus of the current study was 25(OH)D insufficiency, a cut-off of 75 nmol/L was used as a recommended level (i.e., levels of less than 75 nmol/L were categorized as vitamin D insufficient, and those of 75 nmol/L or higher as sufficient).

The data obtained in our study were compared with the data obtained in a Croatian study on vitamin D insufficiency in the general population. In that study, participants were 791 Croatian adults, 660 women and 131 men, mean age 45.5 years. The concentration of circulating 25(OH)D with three threshold values of vitamin D insufficiency (<75 nmol/L, <50 nmol/L and <30 nmol/L) was determined using the immunoassay method[13].

Ethical approval for the study was received from the Sestre milosrdnice University Hospital Center Ethics Committee.

Statistical analyses were performed using the SAS software (version 9.4). The value of p≤0.05 (two-tailed) was considered to be statistically significant. Univariate descriptive statistics, bivariate analyses (table analysis including binomial proportion and odds ratio) and logistic regression (univariate and multivariate) were performed. Results are presented in Tables 1-6.

Results

The study included 371 participants, mean (± standard deviation) age 49.67±17.66 years. The youngest participant was aged 18 and the oldest 91 years, median age 49 years. Residents of inland Croatia were 88.08% of participants, whereas 11.92% were residents of littoral Croatia; there were 64.42% of women and 35.58% of men; 63.61% of patients had their D2 level tested during a period from March to September, and 36.39% during a period from September to March; almost half of patients (47.30%) had a diagnosis of an autoimmune disease (AD), 80.05% did not take vitamin D3 supplementation, 19.95% did take vitamin D supplementation, and 22.10% had a diagnosis of multiple sclerosis (MS). Table 1 shows

Table 1. Vitamin D2 insufficiency according to diagnosis, season, gender, region and supplementation

Variable	Value	25(OH)D insufficiency	n	%
Autoimmune disease	Missing data	Insufficient vitamin D level	1	.
	No	Insufficient vitamin D level	166	85.13
		Sufficient vitamin D level	29	14.87
	Yes	Insufficient vitamin D level	110	62.86
		Sufficient vitamin D level	65	37.14
D2 test date	October	Insufficient vitamin D level	103	76.30
		Sufficient vitamin D level	32	23.70
	March	Insufficient vitamin D level	174	73.73
		Sufficient vitamin D level	62	26.27
Gender	Female	Insufficient vitamin D level	175	73.22
		Sufficient vitamin D level	64	26.78
	Male	Insufficient vitamin D level	102	77.27
		Sufficient vitamin D level	30	22.73
Multiple sclerosis	No	Insufficient vitamin D level	230	79.58
		Sufficient vitamin D level	59	20.42
	Yes	Insufficient vitamin D level	47	57.32
		Sufficient vitamin D level	35	42.68
Region	Littoral Croatia	Insufficient vitamin D level	31	70.45
		Sufficient vitamin D level	13	29.55
	Inland Croatia	Insufficient vitamin D level	245	75.38
		Sufficient vitamin D level	80	24.62
	Missing data	Insufficient vitamin D level	1	
		Sufficient vitamin D level	1	
Vitamin D supplementation	No	Insufficient vitamin D level	243	81.82
		Sufficient vitamin D level	54	18.18
	Yes	Insufficient vitamin D level	34	45.95
		Sufficient vitamin D level	40	54.05

Table 2. Vitamin D2 insufficiency according to age

Analysis variable: age								
Vitamin D2 insufficiency	n	Mean	SD	Minimum	Maximum	Lower quartile	Median	Upper quartile
Insufficient vitamin D level	277	50.14	18.43	18.00	91.00	35.00	49.00	64.00
Sufficient vitamin D level	94	48.26	15.17	22.00	86.00	36.00	48.00	59.00

n = number of patients; SD = standard deviation

percentages of patients with D2 insufficiency according to the subgroups of patients defined above. It can be observed that the percentage of patients with vitamin D2 insufficiency given the AD diagnosis was lower (62.86%) than the percentage of patients without AD diagnosis (85.13%). In the subgroup of patients with MS diagnosis, vitamin D2 insufficiency was detected in only 57.32% of the cases, whereas in the subgroup of neurological patients without MS, 79.58% of the cases were vitamin D2 insufficient. Finally, in the subgroup taking vitamin D3 supplementation, 45.95% of the patients had insufficiency, as opposed to 81.82% of vitamin D2 insufficient patients in the subgroup that did not take the supplements. The percentages of patients with vitamin D2 insufficiency across other subgroups appeared to be only marginally different. Both the mean and median age agreed across the two categories of vitamin D2 insufficiency (Table 2).

In our study, the proportion of neurological patients with 25(OH)D insufficiency (<75 nmol/L) was estimated at 74.66% with 95% confidence interval and low p-value (<0.0001). Table 3 displays 25(OH)D insufficiency status in our study and in a Croatian study in general population. It can be observed that 74.66% of the patients with neurological diseases had vitamin D2 insufficiency, whereas this percentage was somewhat higher (83.82%) in the Croatian adult population.

Patients with AD or MS diagnosis were considerably more likely to have taken vitamin D3 supplements than those without such diagnoses (Tables 4 and 5). The odds ratio of taking D3 supplements was 6.7 times higher for patients with AD diagnosis than for those without it. The odds ratio of taking D3 supplements was 5.6 times higher for patients with MS diagnosis than for those without it.

Table 3. Comparison of 25(OH)D insufficiency in neurological patients and adult Croatian population

	Vitamin D level		
	Inadequate	Adequate	Total
Neurological patients: vitamin D	277 74.66 29.47	94 25.34 42.34	371
General population of Croatia: vitamin D	663 83.82 70.53	128 16.18 57.66	791
Total	940	222	1162

Table 4. Vitamin D3 supplementation in patients with autoimmune disease

Autoimmune disease	Vitamin D supplementation		
	Yes	No	Total
Yes	60 34.29 81.08	115 65.71 38.85	175
No	14 7.18 18.92	181 92.82 61.15	195
Total	74	296	370
Frequency Missing = 1			

Table 5. Vitamin D3 supplementation in patients with multiple sclerosis

Multiple sclerosis	Vitamin D supplementation		
	Yes	No	Total
Yes	37 **45.12** 50.00	45 54.88 15.15	82
No	37 **12.80** 50.00	252 87.20 84.85	289
Total	74	297	371

The diagnosis of MS, diagnosis of AD, and vitamin D3 supplementation were all independently significantly associated with 25(OH)D insufficiency (Table 6). The diagnosis of AD and vitamin D3 supplementation were the most important parameters that influenced 25(OH)D insufficiency (p=0.0023 and p<0.0001, respectively) (Table 6).

The odds ratio of being 25(OH)D insufficient was approximately 2.4 times higher for patients not diagnosed with an AD than for those with AD. In other words, the odds ratio of being vitamin D2 insufficient was 42.2% higher for neurological patients not diagnosed with an AD as opposed to those having such a diagnosis. Likewise, patients not taking vitamin D3 supplements were almost 4 times more likely to be vitamin D2 insufficient as opposed to patients taking the supplements.

Table 6. Association of predictors with 25(OH)D insufficiency in univariate and multivariate logistic regression model

		Model							
		Univariate		Multivariate (without MS)		Multivariate (without AD)		Final multivariate	
Variable	Level of class variable 1 for variable	Estimate	Pr > χ^2	Estimate	Pr > χ^2	Estimate	Pr > χ^2	Estimate	Pr > χ^2
Autoimmune disease	No *vs.* yes	0.6093	**<0.0001**	0.4363	**0.0032**	.	.	0.4183	**0.0023**
Age	.	0.00612	0.3700	-0.00302	0.7135	0.00329	0.6671	.	.
Date	Mar 1, 2021 *vs.* Oct 1, 2021	-0.0685	0.5845	-0.00830	0.9512	-0.0127	0.9253	.	.
Gender	Female *vs.* male	-0.1089	0.3909	-0.0481	0.7267	-0.0375	0.7835	.	.
Multiple sclerosis	No *vs.* yes	0.5329	**<0.0001**	.	.	0.3163	**0.0349**	.	.
Region	Littoral Croatia *vs.* inland Croatia	-0.1251	0.4804	0.0252	0.8976	0.0361	0.8532	.	.
Vitamin D supplementation	No *vs.* yes	0.8333	**<0.0001**	0.6812	**<0.0001**	0.7380	**<0.0001**	0.6866	**<0.0001**

Level of CLASS variable 1 for variable = compared levels of each class (i.e., categorical) variable; Pr > χ^2= the probability of obtaining a χ^2 value greater than the one estimated under the null hypothesis; MS = multiple sclerosis; AD = autoimmune disease

Discussion

In this study, the proportion of neurological patients with 25(OH)D insufficiency was estimated at 74.66% while Colić Baric *et al.* report a somewhat higher proportion of 83.82% in the Croatian adult population[13]. Based on these results, the odds ratio of vitamin D2 insufficiency was by 43% lower for neurological patients than for the Croatian general population. In other words, the odds ratio of the Croatian population being vitamin D2 insufficient was 1.75 times higher than the odds of D2 insufficiency for neurological patients. Although different cut-off points were used across the studies to define vitamin D deficiency and insufficiency, similar results as in our study and Croatian adult population have been reported for the European Union countries and the USA, suggesting that the prevalence of vitamin D2 insufficiency in general population ranges between 75% and 85%[14,15]. Similar results were observed in Southern European and Eastern Mediterranean countries[16], and in a Slovenian study by Hribar *et al.* with a prevalence of 83.3%[17]. Our results show that the proportion of 25(OH)D insufficient patients in the subgroups without AD diagnosis and not taking supplements was 85.13% and 81.82%, respectively. These results suggest that the probability of 25(OH)D insufficiency in neurological patients is higher for patients not taking vitamin D3 supplements and lower for those having a diagnosis of an AD. The lower percentage of patients with 25(OH)D insufficiency across the subgroups with AD or MS diagnosis could be due to the higher percentage of patients with AD (34.29%) or MS (45.12%) taking vitamin D3 supplementation than in the subgroups without AD or MS. Still, 45.59% of patients who were

taking vitamin D3 supplementations in our study were 25(OH)D insufficient. These results support the findings by Carlberg *et al.*[18] and one interventional study[19], where a total of 25% of patients receiving vitamin D3 supplementation showed no adequate response of this laboratory parameter. Similar results have been shown in a study with healthy students[20]. In our study, there was no significant difference in the rate of 25(OH)D insufficient participants according to gender, which was also noted in a study by Colić Barić *et al.*[13] and in one Mediterranean study[21]. In a Chinese study, women were more prone to have 25(OH)D insufficiency[22]. In a Saudi study, significantly lower mean serum 25(OH) D levels were observed in men than in women[23]. Similarly, 25(OH)D deficiency was more common in men than in women in an English study[24]. In our study, there was no significant difference in 25(OH)D insufficiency between the participants from the inland and littoral region, or between the patients tested during October-March and March-October. In a study of patients aged 60 years or older, no seasonal variation was found either[25]. On the contrary, a Danish study showed that women in general had higher median 25(OH)D levels than men, with a higher percentage of 25(OH) D insufficient adults in spring than in autumn in both male and female groups[26]. A review of Central European studies also found vitamin D2 levels to be lower in wintertime than in summertime[27].

There were certain limitations to the comparisons made between the current study results and the results collected for the study conducted for the Croatian population, since, except for the mean age (45.5 in the general population study *vs*. 49.62 in the current study), demographic characteristics of the participants could not be compared. The percentage of 25(OH)D insufficiency in the sample of neurological patients was found to be lower than anticipated. This outcome might be the result of having used a convenience rather than a probability sample. Another possible explanation may be related to the strong association of MS or AD diagnosis with vitamin D3 supplement dosage behavior. To reduce the possible bias in the estimate, it is strongly recommended to adjust the estimate for demographics (age, gender, etc.), but under the assumption that the true distributions of neurological patient population demographic characteristics are known. In that case, it would be possible to compare the prevalence of vitamin D2 insufficiency in neurological patients with that in the general population, while controlling for other variables, i.e., with previous adjustments for the variables that affect the prevalence. The same principle/logic applies to the study results obtained for the Croatian population.

Conclusion

The proportion of neurological patients with 25(OH)D insufficiency was lower than the proportion in the adult Croatian population. There were no significant differences in 25(OH)D insufficiency according to gender, season, and region. Significant differences in 25(OH)D insufficiency were found according to vitamin D3 supplementation and AD diagnosis.

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Sažetak

RETROSPEKTIVNA OPSERVACIJSKA STUDIJA STATUSA VITAMINA D U NEUROLOŠKIH BOLESNIKA

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Glavni cilj istraživanja bio je utvrditi učestalost nedostatne serumske razine 25-hidroksivitamina D u uzorku neuroloških bolesnika te ju usporediti s procijenjenom učestalošću u općoj hrvatskoj populaciji. Sporedni cilj studije bio je utvrditi postoji li moguća značajna razlika u nedostatnoj serumskoj razini vitamina D2 između podskupina bolesnika prema spolu, sezoni testiranja, regiji prebivanja, upotrebi nadomjestaka vitamina D3 i dijagnozi autoimune bolesti. Retrospektivna studija uključila je 371 neurološkog bolesnika tijekom jedne godine. Skupljali su se podaci o spolu, dobi, regiji Hrvatske, dobu godine u kojem je testiranje učinjeno, glavnoj neurološkoj dijagnozi pri otpustu, serumskim razinama 25-hidroksivitamina D u vrijeme hospitalizacije i upotrebi nadomjestaka vitamina D3 prije hospitalizacije. Udio neuroloških bolesnika s nedostatnom razinom 25-hidroksivitamina D (<75 nmol/L) bio je 74,66% (95% CI, p<0,0001), što je niža vrijednost nego u općoj populaciji Hrvatske. Nisu nađene značajne razlike u nedostatnosti 25-hidroksivitamina D u podskupinama s obzirom na spol, sezonu i regiju. Značajne razlike nađene su u podskupinama s obzirom na upotrebu nadomjestaka vitamina D3 i dijagnozu autoimune bolesti.

Ključne riječi: *Nedostatak vitamina D; Multipla skleroza; Autoimuni poremećaji*