

Seasonal variations of 25-OH vitamin D serum levels in Multiple Sclerosis patients with relapse using MRI

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Abstract

An increasing body of evidence suggests that low vitamin D (25-[OH]-D) concentrations is linked to increased activity in multiple sclerosis (MS) patients and MS relapse. Therefore, the current study was aimed to evaluate vitamin D serum concentrations and its possible seasonal variations among MS patients with relapse. This prospective, descriptive study was conducted on patients with MS relapse who referred to the neurology center of Ali ibn Abi Talib Hospital in Zahedan during one year. Magnetic Resonance Imaging (MRI)-Spine was performed for 90 patients and serum samples were collected from patient to measure serum vitamin D levels using RIA KIT. Furthermore, the plaques in each patient's MRI were counted and then recorded. Descriptive and inductive statistics were conducted using statistical software. Our findings indicated a significant correlation between serum vitamin D level and cervical spinal cord plaques ($p = 0.007$, $r = 0.28$), while no association was revealed between serum vitamin D level and number of brain plaque. Furthermore, a significant association was also observed between number of cervical spinal cord plaques and serum vitamin D levels. In addition, a reverse correlation was observed between number of cervical spinal cord plaques and serum vitamin D levels in spring. In autumn, there was a statistically significant relationship between number of brain plaque and serum vitamin D level. Additionally, a statistically significant relationship was found between serum vitamin D levels and number of plaques in winter. Our findings are in agreement with some previous studies that reported conflicting result, where the association of season with the prevalence of relapse cases cannot be verified. Although the mean serum levels of vitamin D are inversely correlated with the incidence of relapses in winter. However, the values obtained in the spring do not confirm such an inverse relationship.

Key Words: Vitamin D, Magnetic Resonance Imaging, Multiple sclerosis, serum.

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Multiple sclerosis (MS) is a demyelinating disorder characterized by selective inflammation and degeneration of central nervous system (CNS) myelin. MS is characterized by a syndrome consisting of inflammation, demyelination and gliosis.¹ MS plaques are typically round or oval, which are seen in the pre-ventricular region or ependymal region, especially in the occipital horn. Although imaging should never be considered alone for diagnosis, the MRI is the most sensitive indicator to find MS plaques.² The causes of the difference in the incidence of multiple sclerosis in the world are uncertain, but genetic and environmental

factors (i.e., sun exposure) may be involved in demyelinating events and relapse probability.³⁻⁵

The incidence of MS is also reported to be remarkably associated with geographical location, when MS is more frequently occurred in both northern and southern areas of the northern and southern hemispheres.⁶⁻⁸ An increasing body of evidence suggests that levels of vitamin D (25-[OH]-D) have latitudinal and seasonal variations, depending on the sunlight exposure.⁴ Vitamin D deficiency has been indicated to be linked to different adverse health outcomes such as MS relapse, and its activity on MRI.⁹⁻¹¹ Although early studies have shown contradictory results,¹²⁻¹⁷ current evidence suggests

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Table 1. Comparison of mean serum vitamin D levels in terms of seasons

Season	Mean (mmol/L)	Standard deviation (SD)	Minimum (mmol/L)	Maximum (mmol/L)	Number	p-value
Spring	28.95	12.52	12.01	59.97	32	
Summer	22.25	12.02	12.06	40.10	15	
Autumn	21.44	10.58	12.16	46.70	26	0.012
Winter	33.85	18.40	12.14	66.45	17	
Total	26.59	13.88	12.07	66.45	90	

seasonal variations of relapse rates.¹⁸⁻²¹ The current study aims to evaluate vitamin D serum concentrations and its possible seasonal variations among MS patients in a neurology center.

Materials and Methods

This prospective, descriptive study was performed on patients with MS relapse who referred to the neurology center of Ali ibn Abi Talib Hospital in Zahedan during one year. All patients with MS were enrolled according to McDonald criteria. Furthermore, all patients with vitamin D supplementation within one year before referral were excluded from the study. In addition, informed consent form was taken from patient for participating in the study. Magnetic Resonance Imaging of spine was performed for 90 patients and a serum sample was obtained from each patient to measure serum vitamin D levels and then kept at -20 ° C. The plaques in each patient's MRI were counted and recorded. At the end of the one-year period, the serum levels of vitamin D in each patient's blood samples were measured by immunoassay method. Accordingly, MS patients were those who were clinically diagnosed with McDonald criteria, including visual, somatosensory and motor evoked potentials, CSF analysis for oligoclonal bands, immunoglobulin G index, and Myelin basic protein. The findings were confirmed by a MS diagnostic

neuroscientist, or the history of two or more MS attacks and a neurologist diagnosis was adequate. MRI was performed immediately after administration of gadolinium DTPA (1.0 mmol / kg). The brain and spinal cord MRI include T2WIS, T1WIS, which T1-WIS with gadolinium contrast infusion (MT) was used due to the consideration of acute lesions. Eighteen brain sections were taken from the brain in three sections including axial, sagittal and coronal with a thickness of approximately 5 mm (field view: 450 x 240 mm; matrix image: 512 x 256 mm, and pixel: 0.94 mm²). In the two axial and sagittal sections, 18 sections were made from the spinal cord with an approximate thickness of about 5 mm (Simpson et al, 2010).⁴ The serum samples were collected from the patients and then kept at -20 ° C until use. Measurement of 25(OH) D serum values was performed using RIA KIT.

Data analysis

One-way ANOVA test was used to compare the serum level of vitamin D in different seasons of the year. To determine the difference in mean of vitamin D in different groups, posthoc Tukey test was used. Spearman's rank correlation coefficient was applied to compare the relationship between serum vitamin D levels and number of brain and cervical spinal cord plaques. Furthermore, chi-square test was used to investigate the

Table 2. Relationship of serum vitamin D levels with brain and cervical spinal cord plaques and total plaques

		Serum Vitamin D levels
Brain plaque	The correlation coefficient	-0.106
	p-value	0.321
	Number of patients	90
Cervical spinal cord plaque	The correlation coefficient	-0.281
	p-value	0.007
	Number of patients	90
Total plaques	The correlation coefficient	-0.188
	p-value	0.077
	Number of patients	90

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Table 3. Comparison of the frequency of brain plaque status based on the seasons

Season	Frequency Yes	Percent Yes	Frequency No	Percent No	Total
Spring	25	78.8	8	21.2	32
Summer	11	73.3	4	26.7	15
Autumn	24	92.6	2	7.4	26
Winter	14	82.4	3	17.6	17
Total	76	82.6	16	17.4	90

Table 4. Comparison of the frequency of cervical spinal cord plaques in terms of seasons

Season	Frequency Yes	Percent Yes	Frequency No	Percent No	Total
Spring	8	25	24	73.3	32
Summer	4	26.7	11	73.3	15
Autumn	8	30.76	18	60.23	26
Winter	5	29.4	12	70.6	17
Total	25	27.77	65	73.33	90

relationship between serum vitamin D level and cervical plaque status. One-sample chi-square test was used to compare the seasonal relapse of the diseases. Spearman's rank correlation coefficient was employed to determine the relationship between serum vitamin D level and total number of plaques. Statistical significance was considered as $p < 0.05$.

Results

Of the 90 patients referred, the number of relapses in the spring, summer, autumn and winter seasons was as follows: 26 (28.88%), 15 (16.66%), 32 (35.55%), and 17 (18.89%). Plaques were ranged between 0 and 6 for brain plaque and 0 to 3 for spinal cord plaques. At the end of the one-year period, the serum vitamin D levels in the blood samples were measured by ELISA method. The mean serum levels of vitamin D in the spring, summer, autumn and winter seasons were 28.95, 44.25, 21.22, 33.85 mmol/L (Table 1). The mean total serum vitamin D level was 26.59 mmol/L, ranging from 12.01 to 66.45 mmol/L, with a standard deviation of 13.88. One-way ANOVA was used to compare the serum level of vitamin D in different seasons of the year. Results showed significant differences in vitamin D levels ($P = 0.012$; Table 1). To determine the difference between mean serum vitamin D level, posthoc Tukey test was used. Results showed that vitamin D level (21.44 mmol/L) was

significantly lower than winter (33.85 mmol/L; $p = 0.018$). Spearman's rank correlation coefficient was used to compare the association between serum vitamin D levels and number of brain and cervical spinal cord plaques in MRI. There was a significant correlation between serum vitamin D level and cervical spinal cord plaques ($p = 0.007$, $r = 0.28$), while no relationship was found between serum vitamin D level and number of brain plaque ($p = 0.32$, $r = -0.10$; Table 2). In addition, there was a significant relationship between the number of brain plaques and cervical spinal cord plaques in MRI ($p < 0.001$; $r = 0.48$). Chi-square test showed no significant relationship between the status of plaque and the season for both brain and cervical spinal cord plaques ($p = 0.33$; $p = 0.99$), (Tables 3 and 4). One-sample chi-square test was used to compare the relapse of the disease according to the seasons. The results showed that the relapse of the disease in various seasons was statistically different ($p = 0.025$), so that the most relapse was seen in the spring (32 cases, 35.55%) and the lowest in the summer (15 cases, 16.66%). Spearman's rank correlation test showed a significant correlation between number of cervical spinal cord plaques and serum vitamin D levels. Furthermore, a reverse correlation between number of cervical spinal cord plaques and serum vitamin D levels was observed in spring ($P = 0.009$; $r = -0.45$; Table 5); however, there was no statistically significant correlation

Table 5. Relationship of serum vitamin D level with brain, cervical spinal cord and total plaques in spring

		Serum Vitamin D levels
Brain plaque	The correlation coefficient	-0.269
	p-value	.136
	Number of patients	32
Cervical spinal cord plaque	The correlation coefficient	-0.457
	p-value	0.009
	Number of patients	32
Total plaques	The correlation coefficient	-0.309
	p-value	0.085
	Number of patients	32

Table 6. Relationship of serum vitamin D level with brain, cervical spinal cord and total plaques in summer

		Serum Vitamin D levels
Brain plaque	The correlation coefficient	-0.047
	p-value	0.869
	Number of patients	15
Cervical spinal cord plaque	The correlation coefficient	-0.221
	p-value	0.428
	Number of patients	15
Total plaques	The correlation coefficient	-0.040
	P-value	0.086
	Number of patients	15

in summer (Table 6). In autumn, there was a statistically significant relationship between number of brain plaque and serum vitamin D level ($p = 0.04$; $r = 0.39$; Table 7). In winter, there was a statistically significant relationship between serum vitamin D levels and number of plaques (i.e., brain and cervical spinal cord plaques, as well as total plaques; $P = 0.36$, $r = -0.5$; $p = 0.001$, $R = -0.71$; $p = 0.001$, $r = 0.71$), (Table 8).

Discussion

Different genetic and environmental factors are capable increasing the risk of MS such latitude, altitude, bioclimatic variables, vitamin D, smoking, infection with Epstein Barr virus (e.g., clouding and ozone levels, etc.,) nevertheless, the impact of each variable alone is not clear.²²⁻²⁷ The correlation of vitamin D levels with MS relapse was reported previously. It has been indicated that vitamin D is capable of modulating both innate and adaptive immune responses. It is capable of modulating Antigen-presenting cells (APCs) that are involving in

reduction of auto-aggressive T cells activation.²⁸⁻³² Vitamin D plays a role in suppressing B cell and T cell differentiation.³³⁻³⁵ The role of VD-associated genes or certain genetic interference with VD is still not fully understood.³⁵ More recent evidence indicated latitudinal and seasonal variations of vitamin D, depending on the sunlight exposure,⁴ therefore, Vitamin D deficiency is potentially involved in the risk of MS, and its relapse.^{9,11,36,37} Our findings showed a seasonal variation in MS relapse with a peak in spring, while the lowest rate was observed in summer and autumn. The high incidence of relapse in the spring and autumn could be due to the involvement of other environmental factors, including infections that are capable of affecting the host's immune system, these interpretation is reminiscent of the results by Ogawa et al., 2004.²⁰ They indicated that total number of attacks was frequently increased in the warmest and coldest months. Heat in hot seasons can be considered as

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Table 7. Relationship of serum vitamin D level with brain, cervical spinal cord and total plaques in autumn

		Serum Vitamin D levels
Brain plaque	The correlation coefficient	-0.398
	p-value	0.044
	Number of patients	26
Cervical spinal cord plaque	The correlation coefficient	-0.271
	p-value	0.181
	Number of patients	26
Total plaques	The correlation coefficient	-0.370
	P-value	0.063
	Number of patients	26

Table 8. Relationship of serum vitamin D level with brain, cervical spinal cord and total plaques in winter

		Serum Vitamin D levels
Brain plaque	The correlation coefficient	-0.510
	p-value	0.036
	Number of patients	17
Cervical spinal cord plaque	The correlation coefficient	-0.710
	p-value	0.001
	Number of patients	17
Total plaques	The correlation coefficient	-0.712
	P-value	0.001
	Number of patients	17

one of the other factors for MS attacks. On the other hand, high occurrence of disease in coldest months could be related to infection disease such as sinusitis.^{20,38,39} Comparison of serum levels of vitamin D in different seasons showed that vitamin D levels were significantly lower in autumn (214.41 mmol/L) than the level of vitamin D in winter (33.58 mmol/L), ($P=0.018$). Serum vitamin D levels did not show statistically significant differences in other seasons. Since this finding is not consistent with the amount of sunlight, comparing the results with other findings is difficult because different criteria have been used of for evaluation of vitamin D level and because serum levels of vitamin D many be affected by various environmental factors, e.g., duration of sunlight and average monthly temperature. In the current study, a significant correlation was found between serum vitamin D level and cervical spinal cord plaques, while no relationship was observed between

serum vitamin D level and number of brain plaque. In addition, a reverse association was revealed between number of cervical spinal cord plaques and low level serum vitamin D in spring. On the other hand, we found a correlation between brain plaque and low level of serum vitamin D in autumn. Taken together, a statistically significant relationship was demonstrated between serum vitamin D levels (low level) and number of plaques in winter but not overall outcomes. As indicated above, the contradiction in these findings eliminates the possibility of providing a logical interpretation in comparison with those of the previously reported investigations, because the nature of diagnostic methods is different for revealing MS exacerbations in studies and results may be affected by various environmental factors (e.g., duration of sunlight, mean monthly temperatures and regional difference of MS prevalence.²⁰ The reasonable seasonal variation of the vitamin D serum concentrations was not

revealed in the present study. In contrast with our results, several other investigations conducted in the northern hemisphere reported strong seasonal variation of the vitamin D serum concentrations in patients with MS.⁴⁰⁻⁴² Our findings are in agreement with some previous studies that reported conflicting results.^{13,14,43,44} A limitation of our study is that it is based upon single clinical center data, which is limited by small sample size. It should be taken into consideration that many reports showed temporal variation in onset of relapses with different diagnostic and inclusion criteria that may affect the findings. Additionally, the definition of recurrence has been different between prospective and retrospective studies. In the present study, the association of season with the prevalence of relapse cases cannot be verified, and may be due to the interference of other environmental factors, including infections. Although the mean serum levels of vitamin D are inversely correlated with the incidence of relapses in winter. However, the values obtained in the spring do not confirm such an inverse relationship. Overall, no significant relationship was found between serum vitamin D levels and number of plaques, bot (brain and cervical spinal cord plaques and total plaques). One limitation of the results here was that this study didn't consider factors such as sun exposure, body mass index and longitudinal vitamin D serum concentrations. Therefore, large multi-center studies, cohorts, and randomized trials are required to determine possible effects of vitamin D in MS.

List of acronyms

APCs - Antigen-presenting cells
CNS - central nervous system
MRI - Magnetic Resonance Imaging
MS - Multiple sclerosis

Authors contributions

SHSS, AM, ARD, MGH, and BHGH conceived the study, performed the experiment, analyzed the data, wrote the paper and helped to editing the manuscript.

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Conflict of Interest

The authors declare no conflicts of interests.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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