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ORIGINAL: Investigating the Relationship Between Vitamin D Status and Multiple Sclerosis; A Case-Control Study

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ABSTRACT

Introduction: Multiple Sclerosis (MS) affects the central nervous system, causing significant impairments in movement and sensory functions. Genetic and environmental factors, including vitamin D deficiency, play crucial roles in MS susceptibility and severity.

Material and Methods: Ninety-six MS patients were examined, and divided into patient (n=48) and control (n=48) groups matched for age and gender. Various parameters like age, disease onset, vitamin D levels, hair color, sunlight exposure, and sunscreen use were analyzed. Disease severity was assessed using the Expanded Disability Status Scale (EDSS) scores.

Results: Statistical analysis revealed significant differences in age, disease onset, serum vitamin D levels, hair color, and sunscreen use at 40 years old between patient and control groups. However, no significant variances were found in gender, age, eye color, and vitamin D levels based on disease type or EDSS scores. Notably, no substantial correlation was established between vitamin D levels and disability severity in MS patients, potentially due to suboptimal vitamin D supplementation.

Conclusion: Patients with MS exhibited significantly lower levels of vitamin D compared to the control group. No significant correlation was found between vitamin D levels and the severity of disability in MS patients. This lack of correlation could be attributed to the suboptimal administration of vitamin D in MS patients.

Introduction

Multiple sclerosis (MS) is a progressive chronic neurological disorder characterized by the demyelination of central nervous system cells, leading to various debilitating symptoms (1, 2). These symptoms encompass sensory disturbances like paresthesia, spinal issues such as spasticity, bladder dysfunction, sexual disorders, cerebellar impairments including tremors and dysarthria, as well as

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vision problems like optic neuritis (3). The disease presents in different forms, including relapsing-remitting, primary-progressive, secondary-progressive, and relapsing-progressive MS, each with distinct clinical courses and symptoms. Diagnosis of MS relies on clinical evidence and paraclinical findings such as magnetic resonance imaging, evoked potentials, and lumbar puncture (4, 5). Advanced tools have emerged in recent decades to assess disease severity and functional decline in MS patients. The Expanded Disability Status Scale (EDSS), is widely employed to evaluate central nervous system function, disease progression, and the efficacy of clinical trial interventions.

Globally, approximately 2.8 million individuals are affected by MS (6), with an estimated prevalence of 37.89 cases per 100,000 people in Asian countries and an average age of onset of 28.21 years (7, 8). In Iran, the prevalence stands at 100 cases per 100,000, with a concerning trend of decreasing patient age. Furthermore, the disease exhibits a higher prevalence among females, with about 60% of affected individuals being women, and a significant proportion of patients experiencing the onset of symptoms between the ages of 18 and 35 (9, 10).

The progression of MS is influenced by various risk factors, including genetic elements such as the HLA-DRB1 15:01 allele, environmental factors like exposure to tobacco smoke and socioeconomic position, Epstein-Barr virus infection, as well as lifestyle factors such as obesity and levels of sunlight exposure and vitamin D (11-19). Although the exact etiology of MS remains elusive, it is widely accepted that both genetic and environmental factors, either independently or synergistically, contribute to the disease's development (20). Notably, the human leukocyte antigen class II gene is a significant genetic factor associated with MS, although it is not the sole cause, as numerous studies have identified non-genetic factors playing a role in the disease's manifestation (15, 21).

Non-genetic factors, particularly vitamin D receptors, have garnered significant attention due to their influence on the acceptance and severity of the disease (22). Vitamin D exerts its impact on gene transcription by interacting with vitamin D receptors on cell membranes (23, 24). Its active form plays a crucial role in regulating the expression of vital genes within the body, and its association with numerous cancers and autoimmune disorders is well-established (25-27). Notably, the central nervous system, including areas such as the hippocampus, cerebellum, brainstem, spinal cord, and cerebral cortex, serves as a target for vitamin D metabolism due to the presence of vitamin D receptors (28). Vitamin D is integral to various immune system functions, including cell proliferation, differentiation, and regulation of immune responses (29). Consequently, vitamin D levels are thought to significantly impact the pathogenesis of MS (30, 31). Studies have shown that the serum vitamin D levels among MS patients are markedly lower than in healthy individuals, although some findings have not supported this distinction (14, 32-35). Studies investigating the relationship between vitamin D levels and MS, especially in Iran, remain limited and yield conflicting results (36, 37). This study aims to bridge this gap by evaluating the correlation between serum vitamin D levels and MS in Mazandaran, Iran, and focuses on assessing the risk of developing the disease by comparing serum vitamin D levels between MS patients and healthy subjects. Additionally, various factors such as gender, age of onset, disease duration, MS type, and EDSS scores will be examined to understand the intricate interconnections between vitamin D levels and the diverse facets of MS.

Methods

In this research, a cohort of 96 patients diagnosed with multiple sclerosis according to the 2017 McDonald criteria by neurologists from Mazandaran University of Medical Sciences was examined. Among these, 48 individuals constituted the patient group, while an equivalent number of 48 healthy individuals, randomly selected from

Bo Ali Hospital in Sari, comprised the control group. Notably, the healthy subjects were chosen at random and had no history of autoimmune diseases. They were carefully matched with the patient group in terms of gender and age. Importantly, individuals in the control group had no personal or familial occurrences of multiple sclerosis, and were devoid of any inflammatory or autoimmune disorders, aligning with the exclusion criteria established for the patients. Ethical guidelines were strictly followed, and written informed consent was obtained from all participants. Demographic data, including age, gender, age at the onset of multiple sclerosis, disease duration, and type of multiple sclerosis, were recorded. Additionally, the degree of disability was assessed using the Expanded Disability Status Scale (EDSS), with scores meticulously documented by trained personnel.

To determine the serum levels of vitamin D, a fasting venous blood sample (5 ml) was collected from each participant. Subsequently, the samples were transferred to sterile 10 ml tubes and analyzed using the 25-Hydroxyvitamin-D3 ELISA Kit for Human Serum. This assay employed a competitive immunological method based on antibody coating. In this process, 25-OH-Vitamin D3 present in the samples competed with the 25-OH-Vitamin D3 bound to the anti-25-OH-Vitamin D3 monoclonal antibody coated on the plate. Following the incubation period, the plates were drained, and HRP-Streptavidin conjugate was introduced into the wells. After another incubation period, the wells were drained, and washed, and an enzyme substrate was added. The enzyme activity observed was inversely proportional to the concentration of 25-OH-Vitamin D3 in the samples. Known standards of 25-OH-Vitamin D3 were tested alongside unknown samples, and the concentration of the unknown samples was determined using a standard curve correlating light absorption with 25-OH-Vitamin D3 concentration. Subsequently, patients were categorized based on their serum vitamin D levels using predefined criteria outlined in the study.

The serum vitamin D levels were classified as deficient (<10 nanograms per milliliter), insufficient (between 10-30 nanograms per milliliter), sufficient (between 30-100 nanograms per milliliter), and toxic (>100 nanograms per milliliter).

The severity and functional status of patients with MS were assessed using the EDSS, a critical measure indicating disease progression. This index ranges from zero, signifying normal neurological condition, to 10, indicating mortality due to MS. Various EDSS scores were utilized in this study to delineate the degrees of patients' functional disability.

The study's inclusion criteria specified that participants had to be 18 years or older. Exclusion criteria involved cardiac, hepatic, and renal failure, pulmonary disease, depression, epilepsy, pregnancy, lactation, hypothyroidism, and various types of multiple sclerosis. Patients previously treated with corticosteroids were excluded, as were individuals who had taken vitamin D supplements, either dietary or medicinal, within the last three months or during pregnancy.

Data was collected using a questionnaire-based tool.

Statistical Analysis

Data entry was conducted using version 24 SPSS software. Descriptive statistics, including percentages, means, standard deviations, and minimum, and maximum values, were used to characterize the variables. The normal distribution of quantitative variables was assessed through the Kolmogorov-Smirnov and Shapiro-Wilk tests. To compare clinical characteristics across different types of multiple sclerosis, ANOVA or Kruskal-Wallis tests were employed, depending on the normal or non-normal distribution of the data, especially for disease duration. Multivariate analysis of patient characteristics related to changes in serum vitamin D levels was performed using linear regression. Independent t-tests were applied for comparing serum levels when the data exhibited normal distribution, while the Mann-Whitney test was used for non-normally distributed data. Chi-square tests,

logistic regression, and Spearman's correlation coefficient were also utilized at a significance level of α=0.05.

Ethical Considerations

In adherence to the Declaration of Helsinki, researchers were obliged to maintain the confidentiality of patient information and identities throughout the study. Before participation, each patient and member of the control group received detailed explanations regarding the research procedures and their voluntary involvement. Informed written consent was obtained from both patients and the healthy control group, affirming their willingness to participate. Participants retained the option to withdraw voluntarily from the study at any stage of the research process.

Results

In our study, the sample comprised 47.9% men and 52.1% women. The distribution of multiple sclerosis subtypes among participants was as follows: 58.3% had RRMS, 33.3% had SPMS, and 4.2% were diagnosed with CIS and PPMS each. The majority of individuals (69.8%) had black hair, while 10.4% had light brown hair, and 19.4% had dark brown hair. Dark brown-eyed individuals constituted 86.5% of the sample, whereas 11.5% had light brown eyes, and individuals with blue or honey eye colors each represented 0.1%. (**Table 1**)

Regarding sunlight exposure and sunscreen use, responses varied among participants. Only 1.77% reported never being exposed to sunlight, 20.8% reported rare exposure, 1.77% experienced pain due to sunlight exposure, and 2.1% were exposed most of the time. In the older age group, 63.5% reported never being exposed to sunlight, 25.0% reported rare exposure, 8.4% reported occasional exposure, and 1.3% reported almost constant exposure. Concerning disability levels measured by the Expanded Disability Status Scale (EDSS), 64.6% had a disability degree of 3.5 or less, 14.6% had a degree between 4 and 5, and 20.8% had a degree of 5.5 or higher. The average age of the participants was 37.99 years (± 8.81), and the average vitamin D serum level was 17.95 ng/mL (± 10.09). (**Table 2**)

Table 1. Frequency of studied subjects according to demographic and clinical findings

		Frequency	Percentage
Gender	Male	46	47.9
	Female	50	52.1
Disease type	CIS	2	4.2
	RRMS	28	58.3
	SPMS	16	33.3
	PPMS	2	4.2
Hair color	Black	67	69.8
	Light Brown	10	10.4
	Dark Brown	19	19.8
Eye Color	Dark Brown	83	86.5
	Light Brown	11	11.5
	Blue	1	1.0
	Hazel	1	1.0
Sun Exposure and Sunscreen Use at Ages 13-19	Rarely	74	77.1
	Sometimes	20	20.8
	Most of the time	2	2.1
Sun Exposure and Sunscreen Use from Age 40 onwards	Rarely	61	63.5
	Sometimes	24	25.0
	Most of the time	8	8.4
	Almost always	3	3.1
Expanded Disability Status Scale (EDSS)	5.3 or lower	31	64.6
	4 to 5	7	14.6
	5.5 or higher	10	20.8

Table 2. Mean and standard deviation of age, age of diagnosis, disease duration and serum level of vitamin D and exposure to sunlight in different seasons in the studied patients

Variable	Count	Mean	Standard Deviation
Age	96	37.9896	8.80609
Age at Disease Diagnosis	96	31.3750	12.59595
Duration of Disease	48	20.9375	30.51292
Sun Exposure in Summer (Ages 13-19)	96	3.8333	1.73913
Sun Exposure in Summer (Age 40 onwards)	96	2.8958	1.67004
Sun Exposure in Winter (Ages 13-19)	96	1.8854	0.98001
Sun Exposure in Winter (Age 40 onwards)	96	1.3959	0.91732
Serum Vitamin D Level	96	17.9464	10.08711

Statistical analyses revealed no significant differences in gender distribution among the studied groups ($p = 0.540$). However, a significant association was found between hair color and multiple sclerosis subtypes ($p < 0.001$), while no significant disparities were observed in eye color ($p = 0.374$). Moreover, no significant differences were found in sunlight exposure and sunscreen usage among individuals aged 13-19 years within the studied groups ($p = 0.319$). In contrast, a significant contrast emerged in sunlight exposure and sunscreen usage among individuals aged 40 and above, showing notable differences within the studied groups ($p = 0.028$). (**Table 3**)

When considering age, there were no significant differences among the studied groups ($p = 0.809$). However, a substantial contrast was identified in the age of disease diagnosis ($p < 0.001$). During summer, no significant differences were found in sunlight exposure among individuals aged 13-19 within the studied groups ($p = 0.316$). Conversely, significant differences were detected in sunlight exposure during summer at the age of 40 within the same groups ($p < 0.001$). Similarly, during winter, no significant differences were observed in sunlight exposure among individuals aged 13-19 within the studied groups ($p = 0.435$). However, a significant contrast was noted in sunlight exposure during winter at the age of 40 within the studied groups ($p < 0.001$). (**Table 4**)

Table 3. Frequency distribution of gender in the studied groups

Variables		Study group count (percent)		Total count (percent)	P-value
		Control	Case		
Gender	Male	21(43.8%)	25(52.1%)	46(47.9%)	.540*
	Female	27(56.3%)	23(47.9%)	50(52.1%)	
Hair Color	Black	26(54.2%)	41(85.4%)	67(69.8%)	.000**
	Light Brown	4(8.3%)	6(12.5%)	10(10.4%)	
	Dark Brown	18(37.5%)	1(2.1%)	19(19.8%)	
Eye Color	Dark Brown	39(81.2%)	44(91.7%)	83(86.5%)	.374**
	Light Brown	7(14.6%)	4(8.3%)	11(11.5%)	
	Blue	1(2.1%)	0(0%)	1(1%)	
	Hazel	1(2.1%)	0(0%)	1(1%)	
Sun Exposure and Sunscreen Use at Ages 13-19	Rarely	34(70.8%)	40(83.3%)	74(77.1%)	.319**
	Sometimes	13(27.1%)	7(14.6%)	20(20.8%)	
	Most of the time	1(2.1%)	1(2.1%)	2(2.1%)	
Sun Exposure and Sunscreen Use from Age 40 onwards	Rarely	24(50%)	37(77.1%)	61(63.5%)	.028**
	Sometimes	18(37.5%)	6(12.5%)	24(25%)	
	Most of the time	4(8.3%)	4(8.3%)	8(8.3%)	
	Almost always	2(4.2%)	1(2.1%)	3(3.1%)	

*Fisher's Exact Test
**Chi-Square Tests

Table 4. Mean and standard deviation of age in the studied groups

	Study Group	Count	Mean	Standard Deviation	P-value
Age	Control	48	37.7708	9.31309	.809*
	Case	48	38.2083	8.36141	
Age at Disease Diagnosis	Control	48	37.4167	9.49094	.000*
	Case	48	25.3333	12.49227	
Sun Exposure in Summer (Ages 13-19)	Control	48	3.9792	1.42156	.316**
	Case	48	3.6875	2.01226	
Sun Exposure in Summer (Age 40 onwards)	Control	48	3.3333	1.29374	.000**
	Case	48	2.4583	1.89016	
Sun Exposure in Winter (Ages 13-19)	Control	48	1.9479	.93536	.435**
	Case	48	1.8229	1.02879	
Sun Exposure in Winter (Age 40 onwards)	Control	48	1.6146	.66236	.000**
	Case	48	1.1773	1.07912	

*Independent Samples Test
**Mann-Whitney Test

Serum vitamin D levels exhibited significant differences among the studied groups ($p < 0.001$), with no significant gender-based disparities ($p < 0.05$). Additionally, the type of disease did not significantly affect the serum vitamin D levels ($p = 0.072$), nor did extensive disability levels measured by EDSS ($p = 0.253$). However, a significant difference was observed in serum vitamin D levels among patients with SPMS and extensive disability degree ($p = 0.021$). (**Table 5**)

Table 5. Mean and standard deviation of serum vitamin D level according to different variables

			Count	Mean	Standard deviation	P-value
Serum Vitamin D Level		Control	48	21.5202	7.22615	.000*
		Case	48	14.3725	11.28533	
Serum Vitamin D Level	Control	Male	21	20.9110	5.71167	.868*
		Female	27	21.9941	8.29247	
	Case	Male	25	13.0172	11.19565	.247*
		Female	23	15.8457	11.44513	
Serum Vitamin D Level		CIS	2	5.6250	3.58503	.072**
		RRMS	28	14.9939	11.13755	
		SPMS	16	16.1000	11.64119	
		PPMS	2	.6000	.25456	
Serum Vitamin D Level		3.5 and lower	31	15.3523	11.21325	.253**
		4 to 5	7	7.1957	3.62051	
		5/5 and higher	10	16.3590	13.76524	
Serum Vitamin D Level	Male	3.5 and lower	16	14.7731	11.24403	.299**
		4 to 5	3	6.0133	5.64781	
		5/5 and higher	6	11.8367	12.98572	
	Female	3.5 and lower	15	15.9700	11.54059	.240**
		4 to 5	4	8.0825	1.58258	
		5/5 and higher	4	23.1425	13.60661	
Serum Vitamin D Level (Sun Exposure and Sunscreen Use in Ages 13-19)	Male	Rarely	41	16.2532	9.85458	.484**
		Sometimes	3	22.7933	11.02253	
		Most of the time	2	14.9000	10.88944	
	Female	Rarely	33	18.8967	10.29595	.782**
		Sometimes	17	19.6882	10.42823	
Serum Vitamin D Level (Sun Exposure and Sunscreen Use in Ages 40 onwards)	Male	Rarely	33	15.9155	10.81997	.605**
		Sometimes	9	19.0089	7.23490	
		Most of the time	4	17.0675	6.78863	
	Female	Rarely	28	19.6846	11.05499	.239**
		Sometimes	15	21.2953	9.53562	
		Most of the time	4	11.6925	4.29432	
		Almost always	3	13.6400	7.76464	

* Mann-Whitney Test
** Kruskal-Wallis Test

Discussion

The statistical analyses of this study revealed significant differences between age, disease diagnosis, serum vitamin D levels, hair color, sunlight exposure, use of sunscreen at 40 years, and the studied groups. However, factors such as gender, age, eye color, sunlight exposure, and sunscreen use between the ages of 13-19 years and at 40 years did not show significant differences in both control and patient groups. Notably, serum vitamin D levels exhibited a statistically significant difference between the control and patient groups. However, no significant difference was observed in vitamin D levels based on gender, disease type, EDSS disability degree, sunlight exposure, and sunscreen use at ages 13-19 and 40 years.

In a related study conducted in 2010, Ghasami et al. investigated the impact of vitamin D3 on nitric oxide levels in MS patients. Vitamin D3 was administered to thirty patients in addition to standard treatment, while thirty patients received only standard treatment. The findings of this study revealed that factors such as age, gender, eye color, sunlight exposure, and nitric oxide concentration did not exhibit statistically significant differences between the treatment and post-treatment (six months later) periods in the two groups of MS patients. Overall, the researchers concluded that the monthly administration of this vitamin over six months had no impact on nitric oxide levels (38). These outcomes contradicted our study findings regarding the measurement of vitamin D types, nitric oxide levels, and the analysis of the studied factors.

In 2017, Zarezadeh and colleagues conducted a case-control study, investigating the serum vitamin D levels in patients with MS in comparison to healthy individuals. Their study aimed to assess the relationship between vitamin D levels and disability in MS patients with 144 participants. In terms of age group and gender distribution, there were no notable differences between the two groups, which contrasts with our research results, particularly concerning the absence of significance related to age. The study revealed that the average vitamin D level in the MS group was lower than the control group. The researchers explained this unexpected result by suggesting that patients with higher disability levels might be prescribed more vitamin D supplements, leading to elevated vitamin D levels (37). This finding aligned with the results of Hejazi et al. in 2014 (39). Additionally, 19.4% of MS patients exhibited toxicity, whereas no cases were observed in the control group, emphasizing significant differences in vitamin D levels between the two groups. Furthermore, our study found a positive and significant correlation between vitamin D levels and the EDSS index in MS patients. Zarezadeh et al.'s study contradicted these results, indicating a significantly higher likelihood of increased disability in patients deficient in vitamin D compared to those with sufficient levels.

In contrast, a study conducted by Bettencourt and colleagues explored serum 25-hydroxyvitamin D levels in MS patients. Their research demonstrated that MS patients had significantly lower vitamin D levels compared to healthy individuals, correlating with higher disability scores (EDSS) and increased MS disease severity (MS). This study highlighted the impact of seasonal changes, with lower vitamin D levels observed in winter. These findings aligned with previous research by Gelfand et al. (2011) (40) and Kirbas et al. (2013). The discrepancy between these studies and Zarezadeh et al.'s findings could be attributed to variations in latitude and sunlight exposure, influencing vitamin D levels in different regions.

In the study titled "The Association of Vitamin D Status with Multiple Sclerosis: A Case-Control Study from Morocco," conducted by Skalli and colleagues in 2018, the link between vitamin D levels and multiple sclerosis (MS) in the Moroccan population was investigated. The study aimed to determine whether vitamin D deficiency was associated with MS and its various

characteristics. Using liquid chromate-graphy-mass spectrometry, the researchers measured the D3(OH)25 metabolite to quantify vitamin D serum levels (DSL) in 113 MS patients and 146 healthy individuals, considering gender and age as factors. The DSLs were then compared between the patient and control groups, and correlations were drawn between DSL and factors such as gender, age of onset, disease duration, type of MS, as well as EDSS and MSSS scores in patients.

Interestingly, the study found that 97.3% of MS patients and 98.6% of controls exhibited hypovitaminosis D (DSL < 30 ng/ml). Although the average DSL in MS patients (11.69±6.69 ng/ml) was slightly lower than that in the control group (12.98±6.58 ng/ml), there was no significant relationship between DSL and MS status (P=0.131). Moreover, no significant associations were observed among different types of MS (RRMS, SPMS, PPMS) concerning DSL, MS, EDSS, or MSSS scores. These results align with prior studies that indicated lower DSLs in MS cases compared to controls, predicting a higher MS risk. Notably, the study indicated that DSLs were not linked to disease risk or MS type but were associated with disability and disease severity, suggesting a limited role of hypovitaminosis D in the pathogenesis of MS in the Moroccan population. The authors suggested the need for further research to comprehensively explore the vitamin D pathway's role in MS, potentially paving the way for innovative prevention and treatment strategies (Skalli et al., 2018) (41).

In another study conducted by Fayyazi and colleagues in 2018, titled "Sunlight Exposure and Vitamin D: Implications for Disability and Progression in Multiple Sclerosis," the relationship between sunlight exposure, vitamin D intake, MS disability, and fatigue was explored. This study included 197 MS patients from Kashani Hospital of Isfahan's MS clinic. Researchers assessed sun exposure, vitamin D intake, physical activity, and fatigue levels in these patients across different MS phenotypes.

The findings revealed higher disability status scale and fatigue scale scores in primary-progressive MS (PPMS) and secondary-progressive MS (SPMS) groups compared to the relapsing-remitting MS (RRMS) group. Interestingly, RRMS patients exhibited higher scores in both sun exposure and physical activity compared to SPMS and PPMS groups. Furthermore, a negative correlation was noted between fatigue scale scores and sunlight exposure in RRMS patients. Surprisingly, vitamin D intake from the diet did not significantly differ among the groups.

In summary, the study highlighted that RRMS patients had higher sunlight exposure, indicating a potential link between sunlight exposure and reduced fatigue in this subgroup. Conversely, PPMS patients exhibited lower sunlight exposure. The study underscored the need for more extensive research with larger sample sizes and robust methodologies to validate these findings further (39).

In their 2021 review, Wesnes and colleagues delved into a study exploring the impact of modified lifestyle factors on the 10-year clinical disability progression in patients with Multiple Sclerosis (MS) (80). This prospective study, involving 88 MS patients, meticulously analyzed serum 25-hydroxyvitamin D (25(OH)D), serum cotinine levels (indicative of nicotine exposure), and BMI between 2004 and 2008. A subsequent follow-up study in 2017, involving 80 participants, utilized the Expanded Disability Status Scale (EDSS) to discern the relationship between these lifestyle factors and EDSS changes over the decade. The results indicated a notable seasonal association between higher vitamin D levels and reduced 10-year EDSS progression. Surprisingly, no clear correlation was found between BMI and cotinine levels. However, this study revealed a consistent and significant link between elevated vitamin D levels and diminished 10-year disability progression, independent of potential confounding factors related to lifestyle and disease status. Importantly, the researchers noted that a more extended

observation period might be necessary to capture vitamin D's potential effects on physical and cognitive disability scores (37).
In a parallel study conducted in 2017, Rito et al. investigated the relationship between vitamin D and disability in relapsing-remitting multiple sclerosis (RRMS) among 50 Mexican patients (81). Assessing factors like EDSS score, Progression Index (PI), and Annual Recurrence Rate (ARR) during the summer, the study yielded intriguing results. While no significant correlation was identified between vitamin D levels and EDSS, ARR, PI, or disease duration, a modest negative association between vitamin D levels and EDSS was observed specifically in women. The researchers hypothesized that this intricate relationship between vitamin D levels and MS disability might be influenced by the interplay of genetic expression and environmental factors. They highlighted the polymorphic variation in the vitamin receptor's genetic expression, which affects the immune system, suggesting a need for further genetic and environmental investigations in MS patients (38).
Furthermore, it is noteworthy that despite residing in sunny regions, the studied cases exhibited surprisingly low levels of vitamin D. This paradoxical situation was attributed, in part, to the higher pigment content in the skin of populations living in tropical regions, which interferes with vitamin D production. In the context of relapsing-remitting multiple sclerosis (RRMS), disease activity is a crucial factor influencing disability. Nonetheless, external factors such as the frequency of relapses, their treatment, the efficacy of disease-modifying treatments, and lifestyle elements including a balanced diet, physical activity, and mental well-being also play pivotal roles (38).
The study's limitations, pertinent for future research, include the necessity to explore vitamin D intake from both food and supplements among participants. Additionally, it is advisable to consider evaluating the surface area exposed to sunlight, given its vital role in vitamin D synthesis. One of the notable drawbacks of this study is the relatively small sample size, indicating a need for larger-scale investigations in subsequent studies.

Conclusion

Our study revealed significant variances in MS susceptibility related to factors like age, disease diagnosis, vitamin D levels, hair color, sunlight exposure, and sunscreen use at 40 years old. However, gender, disease type, extensive disability level, and sunlight exposure at specific ages did not significantly impact vitamin D levels. These findings contribute valuable insights into the complex genetic and environmental factors influencing MS severity and susceptibility.

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Conflicts of interest

The authors declare they have no conflicts of interest and have no financial interest related to any aspect of the study.

Authors' contributions

All authors contributed to the study's design, data analysis, manuscript drafting, and critical revisions, approved the final version for submission, and accepted accountability for the work.

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