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ORIGINAL PAPER

VITAMIN D DEFICIENCY, DISABILITY, AND INFLAMMATORY BIOMARKERS IN MULTIPLE SCLEROSIS: A RETROSPECTIVE CROSS-SECTIONAL STUDY

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Abstract

Introduction. Multiple sclerosis (MS) is a chronic neuroinflammatory disease influenced by various environmental factors. Among these, vitamin D deficiency has emerged as a potential modifiable risk factor that may be associated with immune dysregulation and clinical severity in patients with MS.
The objective of the study was to explore the association between vitamin D status, inflammatory hematological parameters and clinical status of MS patients.
Materials and methods. A retrospective cross-sectional study was conducted. A total of 605 patients with MS were identified from two university hospitals, among whom 545 had available serum 25-hydroxyvitamin D measurements and were included in the analyses. Serum vitamin D levels, hematological markers, clinical symptoms and Expanded Disability Status Scale (EDSS) scores were analysed. The patients were

Résumé

Carence en vitamine D, handicap et biomarqueurs inflammatoires dans la sclérose en plaques: une étude transversale rétrospective

Introduction. La sclérose en plaques (SEP) est une maladie neuro-inflammatoire chronique influencée par divers facteurs environnementaux. Parmi ceux-ci, la carence en vitamine D est apparue comme un facteur de risque potentiellement modifiable, susceptible d'être associé à un dérèglement immunitaire et à une sévérité clinique accrue chez les patients atteints de SEP.
L'objectif de l'étude était d'explorer l'association entre le statut en vitamine D, les paramètres hématologiques inflammatoires et l'état clinique des patients atteints de SEP.
Matériels et méthodes. Une étude transversale rétrospective a été réalisée. Au total, 605 patients atteints

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classified into three groups according to their vitamin D status.

Results. The study mean age of the patients was 37±11 years. Distribution by sex: 434 (71.7%) were women and 171 (28.3%) were men (sex ratio 2.5). Patients with vitamin D deficiency presented more frequently neuromuscular symptoms and significantly higher EDSS scores ($p < 0.0001$). These patients showed an elevated neutrophil-to-lymphocyte ratio (NLR), marked lymphopenia and eosinophilia, all of which were significantly correlated with low vitamin D levels. A strong negative correlation was observed between serum vitamin D and EDSS score ($r = -0.401$, $p < 0.0001$).

Conclusions. Vitamin D deficiency was associated with higher disability scores and an inflammatory hematological profile in patients with MS. Because of the retrospective cross-sectional design, these findings should be interpreted as associations rather than evidence of causality.

Keywords: 25-hydroxyvitamin D, neutrophil-to-lymphocyte ratio, lymphopenia, eosinophilia, immune biomarkers.

List of abbreviations:
25(OH)D – 25-hydroxyvitamin D
ANOVA – Analysis of Variance
EDSS – Expanded Disability Status Scale
HBP – high blood pressure
MS – multiple sclerosis
NLR – neutrophil-to-lymphocyte ratio
PLR – platelet-to-lymphocyte ratio
PPMS – primary progressive multiple sclerosis
PRMS – progressive relapsing multiple sclerosis
PS – pyramidal syndrome
RRMS – relapsing-remitting multiple sclerosis
SD – standard deviation
SPMS – secondary progressive multiple sclerosis

de SEP ont été identifiés dans deux centres hospitaliers universitaires; parmi eux, 545 disposaient de mesures de la 25(OH)D sérique et ont été inclus dans les analyses. Les taux sériques de vitamine D, les marqueurs hématologiques, les symptômes cliniques et les scores Expanded Disability Status Scale (EDSS) ont été analysés. Les patients ont été répartis en trois groupes selon leur statut en vitamine D.

Résultats. L'âge moyen des patients inclus dans l'étude était de 37±11 ans. Répartition par sexe: 434 (71,7 %) étaient des femmes et 171 (28,3 %) des hommes (sex-ratio de 2,5). Les patients présentant une carence en vitamine D manifestaient plus fréquemment des symptômes neuromusculaires et affichaient des scores EDSS significativement plus élevés ($p < 0,0001$). Ces patients présentaient un rapport neutrophiles/lymphocytes (RNL) élevé, ainsi qu'une lymphopénie et une éosinophilie marquées; ces paramètres étaient tous significativement corrélés à de faibles taux de vitamine D. Une forte corrélation négative a été observée entre le taux de vitamine D et le score EDSS ($r = -0,401$, $p < 0,0001$).

Conclusions. La carence en vitamine D était associée à des scores de handicap plus élevés et à un profil hématologique inflammatoire chez les patients atteints de SEP. En raison de la conception transversale rétrospective de l'étude, ces résultats doivent être interprétés comme des associations plutôt que comme la preuve d'un lien de causalité.

Mots-clés: 25-hydroxyvitamine D, rapport neutrophiles/lymphocytes, lymphopénie, éosinophilie, biomarqueurs immunitaires

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease affecting the central nervous system, leading to deterioration of myelinated neurons and severe neurological, cognitive and physical disorders[1]. MS is characterized by the formation of plaques in the central nervous system, especially in the white matter of the brain and spinal cord, accompanied by inflammation, demyelination, and axonal damage[2]. Considered an autoimmune disease, it is caused by autoreactive immune cells that cross the blood-brain barrier and attack the central nervous system. Both T and B lymphocytes play critical roles in the pathogenesis of the disease, with T cells mediating direct cytotoxicity and B cells producing autoantibodies and amplifying immune activation[3,4].

It is estimated that approximately 2.8 million people worldwide live with MS, which mainly affects young adults, with a marked female predominance (3:1)[5]. The prevalence of MS increases with latitude, suggesting a key role for environmental factors such as sun exposure and vitamin D levels[6].

Vitamin D is an essential immunomodulator involved in the regulation of innate and adaptive immunity, and its receptors are expressed in multiple brain regions, including the hippocampus, hypothalamus, thalamus, and neocortex[1]. Low serum 25-hydroxyvitamin D levels are associated with increased risk of neurological disability, including MS[7]. In addition,

vitamin D may influence systemic inflammation, reflected in hematological markers such as the neutrophil-to-lymphocyte ratio (NLR), a biomarker of pro-inflammatory states linked to disease severity[8].

Previous studies assessing the impact of vitamin D on MS progression have been limited by small cohorts and incomplete analysis of inflammatory markers such as NLR, preventing definitive conclusions on its role in disease progression. Given the well-established immunomodulatory properties of vitamin D and the growing importance of NLR as a prognostic marker in neuroinflammatory diseases, we hypothesized that vitamin D deficiency was associated with higher NLR, lymphopenia and greater disability.

The objective of the study was to evaluate the association between vitamin D status, inflammatory hematological markers and disability scores in a large Algerian cohort of patients with MS.

Materials and methods

This retrospective cross-sectional study included 605 consecutive patients diagnosed with MS according to the 2017 revised McDonald criteria[9], followed at the neurology departments of Sidi Bel Abbes University Hospital and Oran University Hospital (Algeria), between January 2010 and April 2025. Only patients with available serum 25(OH)D measurements (n = 545) were included in analyses evaluating vitamin D status.

The inclusion criteria were: confirmed MS diagnosis, available serum 25(OH)D measurement, and EDSS evaluation within ±3 months of the blood draw. The exclusion criteria included recent infections (within 4 weeks), corticosteroid treatment within 4 weeks, pregnancy, and concurrent autoimmune disease other than MS.

While clinical and hematological data were retrospectively collected from medical records, serum 25(OH)D concentrations were measured specifically for this study. All procedures, including the measurement of vitamin D levels and the use of patients' data, were approved by the local ethics committee (approval no. 27, April 14, 2025) and conducted in accordance with the Declaration of Helsinki.

The parameters taken into consideration included demographic and clinical data, in particular age, gender, type of MS, symptoms, and EDSS score. Additionally, serum levels of vitamin D, hemoglobin, hematocrit, platelets, and leukocyte subpopulations (neutrophils, lymphocytes, basophils, eosinophils, and monocytes) were analyzed. Neutrophil/lymphocyte and platelet/lymphocyte ratios were also calculated as indicators of inflammatory status. Vitamin D status was classified as deficient (<20 ng/mL), insufficient (20-29 ng/mL), or normal groups (30-100 ng/mL).

Serum concentrations of 25-hydroxyvitamin D [25(OH)D] were determined using the VIDAS® 25 OH Vitamin D Total assay (bioMérieux) performed on the VIDAS® automated analyzer. This method is based on an enzyme-linked fluorescent assay (ELFA). The assay is calibrated according to the ID LC/MS/MS reference method (SRM 2972).

Vitamin D levels were available for only 545 of the 605 patients, and all analyses related to vitamin D status were therefore performed exclusively on this subgroup.

Hemoglobin, hematocrit, platelets, and leukocyte subpopulations (neutrophils, lymphocytes, basophils, eosinophils, and monocytes) were measured in whole blood collected in EDTA tubes. The analysis was performed using a Coulter® hematology analyzer (DxH 800, Beckman Coulter Inc., Brea, CA, USA), which combines electrical impedance technology (Coulter principle) with optical analysis for leukocyte differentiation.

The neutrophil-to-lymphocyte ratio was determined based on the absolute concentrations of neutrophils and lymphocytes obtained from the white blood cell count performed at the time of patient admission.

The platelet-to-lymphocyte ratio (PLR) was determined based on the absolute platelet and lymphocyte counts obtained from the blood count performed upon admission to the department.

Statistical analyses

Statistical analyses were performed using SPSS version 22.0 (IBM Corporation, Chicago, IL, USA). Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed variables are presented as mean±standard deviation (SD), while non-normally distributed variables are presented as median (interquartile range, IQR). Categorical variables are presented as frequencies and percentages.

Comparisons between vitamin D groups (normal, insufficient, and deficient) were performed using ANOVA for normally distributed continuous variables or the Kruskal–Wallis test for non-normally distributed variables. Pearson's chi-square (χ^2) test was used for categorical variables.

Associations between serum 25(OH)D levels and EDSS, lymphocyte count, eosinophil count, and neutrophil-to-lymphocyte ratio (NLR) were assessed using Spearman's correlation. A two-sided p-value < 0.05 was considered statistically significant.

Table 1. Demographic and clinical data of multiple sclerosis patients

Variables	*Modalities/ Statistics*	*Normal (n=48)*	*Insufficiency (n=35)*	*Deficiency (n=462)*	*p-value*
Age (years)	Mean ± SD	34.31±12.33	37.51±9.63	36.73±11.2	0.31
	17-27	15(31.3%)	05(14.3%)	117(25.3%)	0.57
	28-38	14(29.2%)	13(37.1%)	117(25.3%)	
	39-49	13(27.1%)	14(40%)	158(34.2%)	
	50-60	06(12.5%)	03(8.6%)	57(12.3%)	
	61-71	00(00%)	00(00%)	13(2.8%)	
Gender	Women	34(70.8%)	19(54.3%)	321(73%)	0.058
	Men	14(29.2%)	16(45.7%)	141(27%)	
Initial clinical presentation	Muscle weakness	28(58.3%)	23(65.7%)	355(76.8%)	**0.008**
	Fatigue	3(6.3%)	2(5.7%)	29(6.3%)	0.99
	Visual disorders	22(45.8%)	15(42.9%)	186(35.6%)	0.28
	Sphincter disorders	6(12.5%)	2(5.7%)	70(13.7%)	0.42
	Gait disorders	6(12.5%)	7(20%)	126(24.1%)	0.16
	Balance disorders	2(4.2%)	7(20%)	116(22.2%)	**0.013**
	Vertigo	5(10.4%)	5(14.3%)	25(5.4%)	**0.024**
Clinical syndromes	PS	11(22.9%)	11(31.4%)	166(31.8%)	0.44
	CS	44(91.7%)	31(88.6%)	439(84.1%)	0.30
	SCS	4(8.3%)	3(8.6%)	26(5%)	0.43
	Med. inv.	00(00%)	2(5.7%)	11(2.1%)	0.20
Comorbidities	T2DM	1(2.1%)	00(00%)	5(1%)	0.62
	HBP	2(4.2%)	00(00%)	8(1.5%)	0.28

Notes: Values are expressed as n (%) except where indicated (mean ± SD). P-value for means: ANOVA. Significant p-values shown in bold ($p < 0.05$).
Abbreviations: PS – pyramidal syndrome; CS – cerebellar syndrome; SCS – spinal cord syndrome; Med. Inv. – medullary involvement; T2DM – type 2 diabetes mellitus; HBP – high blood pressure.

Results

The patients with available vitamin D measurements (n = 545) were classified into three groups according to serum vitamin D concentration: normal, insufficient, and deficient. Normally distributed continuous variables are presented as mean±SD, non-normally distributed variables as median (IQR), and categorical variables as frequencies and percentages. Comparisons between groups were performed using ANOVA or Kruskal–Wallis test for continuous variables and Pearson's χ^2 test for categorical variables.

Table 1 presents the distribution of patients with MS according to vitamin D status, age, gender, initial clinical manifestations, neurological syndromes, and comorbidities. The average age was similar across all three groups, with no statistically significant difference (ANOVA, p = 0.31). Regarding gender, women predominated in all categories, with a slight decrease in the "insufficient" group compared to the "normal" and "deficient" groups, although this difference was not significant (p = 0.058).

Concerning the initial clinical manifestations, significant differences were observed between vitamin D groups for muscle weakness (p = 0.008), balance disorders (p = 0.013), and vertigo (p = 0.024). No significant differences were observed between the three groups for fatigue, visual disorders, sphincter disorders, or gait disorders (p > 0.05).

Cerebellar syndrome was observed in more than 84% of patients, followed by pyramidal syndrome (~30%). Spinal cord syndrome and medullary involvement were less frequent. No significant association was observed between these neurological syndromes and vitamin D levels (p > 0.05).

Finally, type 2 diabetes and high blood pressure were uncommon in the study population and did not differ significantly between vitamin D groups (p > 0.05).

Table 2 summarizes the relationship between vitamin D status, the degree of disability assessed by EDSS, and the clinical forms of MS.

EDSS scores differed significantly between vitamin D groups (Kruskal–Wallis test, p < 0.0001). Patients with vitamin D deficiency had significantly higher EDSS scores (mean 3.40±1.62) compared to those with insufficiency (2.18±1.89) and normal levels (1.25±1.53). The distribution of EDSS categories differed significantly between groups (p < 0.0001). In the deficient group, 49% of patients were in the 2–3.5 EDSS category, compared with 28.6% in the

Table 2. Expanded disability status scale scores and forms of multiple sclerosis

Variables	*Modalities/Statistics*	*Normal (n=48)*	*Insufficiency (n=35)*	*Deficiency (n=462)*	*p-value*
EDSS	Mean ± SD	1.25±1.53	2.18±1.89	3.40±1.62	**<0.0001**
	0 to 1.5	35(72.9%)	19(54.3%)	51(10.9%)	**<0.0001**
	2 to 3.5	09(18.8%)	10(28.6%)	226(49%)	
	4 to 5.5	03(6.3%)	03(8.6%)	130(28.2%)	
	6 to 6.5	00(00%)	01(2.9%)	37(8%)	
	7 and more	01(2.1%)	02(5.7%)	18(3.8%)	
Clinical form	RRMS	42(87.5%)	28(80%)	395(85.5%)	0.41
	PPMS	00(00%)	03(8.6%)	14(3.03%)	
	SPMS	04(8.3%)	03(8.6%)	36(7.8%)	
	PRMS	02(4.2%)	01(2.9%)	17(3.68%)	

Notes: Values are expressed as n (%) except where indicated (mean ± SD). P-value for means: ANOVA. Significant p-values shown in bold ($p < 0.05$).
Abreviations: EDSS – Expanded Disability Status Scale; SD – standard deviation; RRMS – relapsing–remitting multiple sclerosis; PPMS – primary progressive multiple sclerosis; SPMS – secondary progressive multiple sclerosis; PRMS – progressive relapsing multiple sclerosis.

Table 3. Hematological parameters of multiple sclerosis patients

Variables	*Units*	*Normal (n=48)*	*Insufficiency (n=35)*	*Deficiency (n=462)*	*p-value*
Hemoglobin	(g/dL)	13.45 ± 1.37	13.48±1.88	13.77±1.40	0.161
Hematocrit	(%)	39.49±5.96	40.84±3.43	41.02±5.15	0.783
Platelets	($\times 10^9$/L)	277.48±51.01	260.11±49.57	263.61±49.53	0.156
Neutrophils	($\times 10^9$/L)	4.16±1.23	4.05±1.02	4.12±1.42	0.942
Lymphocytes	($\times 10^9$/L)	1.78±0.62	1.48±0.39	1.47±0.52	**0.001**
Basophils	($\times 10^9$/L)	0.032±0.019	0.034±0.018	0.038±0.091	0.886
Eosinophils	($\times 10^9$/L)	0.29±0.30	0.35±0.17	0.40±0.15	**p<0.001**
Monocytes	($\times 10^9$/L)	0.45±0.18	0.52±0.20	0.60±0.23	**0.027**
NLR	Ratio	2.34±0.44	2.74±0.18	2.80±1.37	**p<0.001**
PLR	Ratio	155.89±69.23	175.75±81.55	179.33±84.14	0.627

Notes: Values are expressed as n (%) except where indicated (mean ± SD). P-value for means: ANOVA. Significant p-values shown in bold ($p < 0.05$).
Abbreviations: NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; ANOVA – analysis of variance.

insufficiency group. In the deficient group, 28.2% of patients were in the 4–5.5 EDSS category, compared with 6.3% in the normal group.

Regarding the clinical forms of MS, the majority are represented by the relapsing-remitting form in all groups (>80%), with no significant difference ($p = 0.41$).

Table 3 shows the hematological parameters according to vitamin D status. A significant decrease in lymphocytes was observed in groups with vitamin D insufficiency and deficiency (ANOVA, $p = 0.001$). In addition, eosinophil and monocyte counts were significantly higher in vitamin D-deficient patients ($p < 0.05$). The NLR differed significantly between the vitamin D groups ($p = 0.000$), while PLR did not ($p = 0.627$). Other parameters, including hemoglobin, hematocrit, platelets, neutrophils, and basophils, did not differ significantly between groups ($p > 0.05$).

In addition to these group comparisons, correlation analyses revealed several significant associations. Vitamin D levels showed a strong negative correlation with EDSS scores ($r = -0.401$, $p < 0.0001$), indicating that lower vitamin D concentrations were associated with greater disability. A significant positive correlation was found between vitamin D levels and lymphocyte counts ($r = 0.115$, $p = 0.005$), while significant negative correlations were observed with eosinophil counts ($r = -0.234$, $p < 0.0001$) and with the NLR ($r = -0.436$, $p < 0.0001$).

Discussion

In this study, we aimed to examine the relationship between vitamin D levels, hematological inflammatory parameters and clinical status in Algerian patients with multiple sclerosis. A total of 605 patients

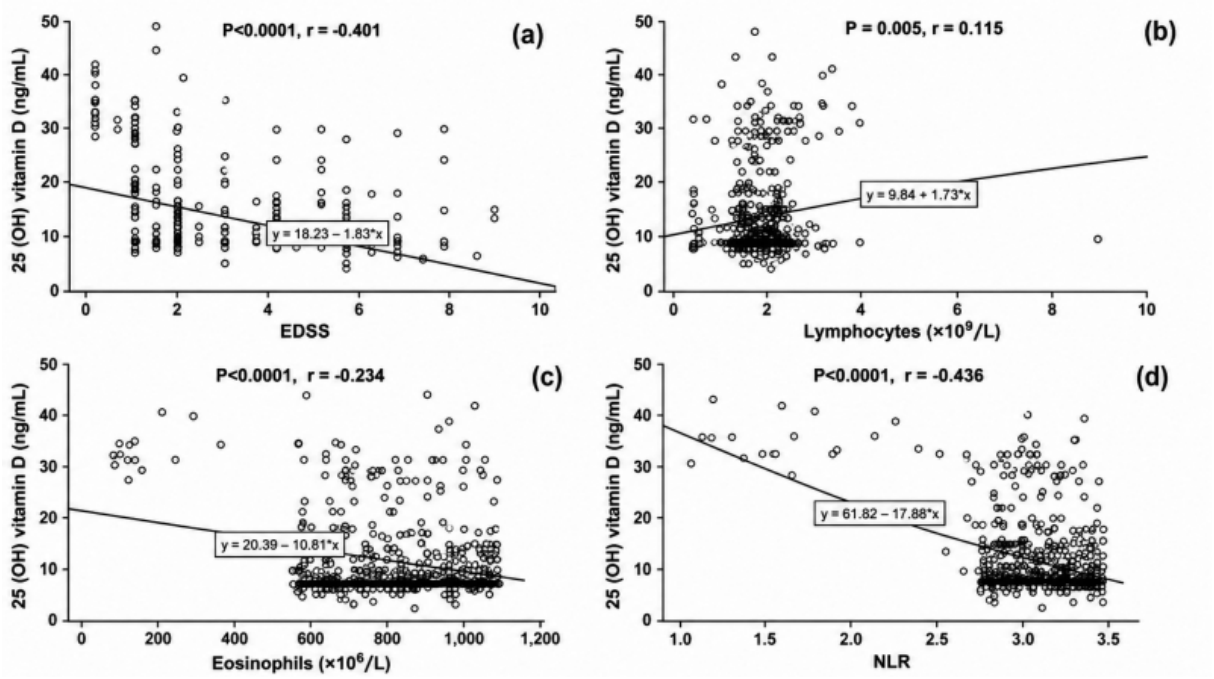


Figure 1. Correlations between serum 25-hydroxyvitamin D [25(OH)D] levels and clinical and inflammatory parameters in patients with multiple sclerosis.

Legend: Scatter plots showing the correlations between serum 25(OH)D levels and (a) Expanded Disability Status Scale (EDSS) score ($r = -0.401$, $p < 0.0001$), (b) lymphocyte count ($r = 0.115$, $p = 0.005$), (c) eosinophil count ($r = -0.234$, $p < 0.0001$), and (d) neutrophil-to-lymphocyte ratio (NLR) ($r = -0.436$, $p < 0.0001$). Solid lines represent linear regression.

with MS were recruited during this study. No significant differences were observed in age between the three vitamin D groups (mormal: 34.31±12.33 years; insufficiency: 37.51±9.63 years; deficiency: 36.73±11.21 years), consistent with the findings of Alelyani et al.[10].

Regarding gender, our results reveal a female predominance in all groups, with a slight decrease in the "insufficient" group (54.3%) compared to the "normal group" (70.8%) and the "deficiency" group (73%). This trend approached statistical significance ($p = 0.058$), and is in line with previous studies suggesting a possible influence of gender on vitamin D status[11,12].

The analysis of clinical manifestations revealed statistically significant differences according to vitamin D status. Deficient patients exhibited a higher prevalence of muscle weakness ($p = 0.008$), balance disorders ($p = 0.013$), and vertigo ($p = 0.024$). While vitamin D deficiency may influence neuromuscular and vestibular function, the exact mechanisms were not directly assessed in this study, and these associations should be interpreted cautiously.

Indeed, the pronounced muscle weakness observed in the deficient group may be related to disturbances in calcium metabolism. Vitamin D deficiency has been reported to reduce intestinal calcium absorption to only 10-15% (compared to 30-40% under normal conditions), thereby altering calcium availability for muscle contraction and neuromuscular transmission. The resulting relative hypocalcemia stimulates the secretion of parathyroid hormone[13]. However, chronic parathyroid hormone elevation can induce deleterious effects on the musculoskeletal system, including proximal myopathy and impaired muscle function, as demonstrated in studies on deficiency myopathies[14].

Furthermore, the observed balance disorders and vertigo may be related to a role of vitamin D in vestibular function. Recent studies report expression of the vitamin D receptor and metabolizing enzymes in the inner ear (vestibular region) and show VDR/CYP27B1 expression in human brain tissue, findings that make a modulatory role of vitamin D in sensorimotor pathways plausible. However, direct evidence for VDR expression specifically in human vestibular nuclei remains limited. Therefore, vitamin D deficiency could plausibly aggravate pre-existing neurological deficits in MS patients, but this hypothesis requires further mechanistic confirmation[15-17].

In contrast, our study found no statistically significant association between vitamin D status and clinical forms of MS. The relapsing-remitting form was predominant (>80%) across all groups, with no significant variation. This observation contrasts with some previous studies, which reported significant

associations. For instance, Spiezia and al. found that vitamin D concentrations were higher in relapsing-remitting forms than in progressive forms, potentially reflecting a modulatory role of vitamin D in the early inflammatory mechanisms of the disease[18]. This divergence highlights the complexity of the interactions between vitamin status and the clinical heterogeneity of MS, which may be influenced by genetic predisposition, environmental factors, or disease duration.

Additionally, comorbidities such as type 2 diabetes and hypertension were rare in our cohort (less than 2% of cases), and their distribution was not significantly influenced by vitamin D concentrations. Our results differ from several previous studies suggesting that comorbidities play an unfavorable role in MS progression and vitamin D status[19,20,21].

In our analysis, a negative linear correlation was identified between serum vitamin D concentrations and EDSS scores ($r = -0.401$, $p < 0.0001$), indicating that low vitamin D levels were associated with more severe disability progression. These findings are consistent with several recent studies, including a meta-analysis by Sintzel et al., which showed that sufficient vitamin D levels were associated with a reduced risk of relapses and slower disability progression in patients with relapsing-remitting multiple sclerosis (RRMS)[22]. In addition, the study by Bettencourt et al. reported a negative linear correlation between serum 25-hydroxyvitamin D levels and EDSS scores ($r = -0.293$, $p < 0.0001$) in a cohort of MS patients from northern Portugal[23]. Similarly, a cross-sectional study from the EVIDIMS randomized trial by Bäcker-Koduah et al. indicated that lower vitamin D levels are associated with greater neurological disability in patients with MS[24]. Furthermore, a prospective study by Galus et al. confirmed that high levels of 25(OH)D were predictive of better clinical outcomes, supporting the hypothesis of a neuroprotective effect of vitamin D in MS[25].

Regarding hematological parameters, vitamin D-deficient patients exhibited a significantly higher NLR and lower lymphocyte counts. In addition, serum vitamin D levels were positively correlated with lymphocyte counts ($r = 0.115$, $p = 0.005$), supporting the previously reported anti-inflammatory role of vitamin D[26-28]. Additionally, significant differences in eosinophil ($p < 0.0001$) and monocyte ($p = 0.027$) counts were observed between groups, suggesting an association between vitamin D status and immune cell distribution. In contrast to NLR, PLR showed no significant association with vitamin D status. This may reflect its lower sensitivity as an inflammatory marker in MS, as previously observed in neuroinflammatory disorders. However, the present study did not assess specific cytokines, immune cell phenotypes, or other molecular mechanisms, and therefore mechanistic interpretations should be made cautiously.

In our study, lower serum vitamin D levels were associated with higher eosinophil counts. Vitamin D is known to exert immunomodulatory effects, including the suppression of Th2-mediated responses and the regulation of eosinophil survival and activation. Deficiency in vitamin D may lead to unchecked Th2 activity, resulting in increased circulating eosinophils. Similar inverse relationships between vitamin D status and eosinophil counts have been reported in asthma, allergic diseases, and certain autoimmune conditions. In the context of MS, although the disease is primarily driven by Th1/Th17 responses, elevated eosinophils may indicate an underlying immune imbalance associated with vitamin D deficiency, potentially contributing to disease activity and inflammation[29].

Given the retrospective design of this study, the results demonstrate associations rather than causality. Vitamin D deficiency cannot be considered a direct causal factor in MS progression based on these data alone, but the observed relationships support the potential clinical relevance of monitoring vitamin D levels in patients with MS.

This study has several limitations that should be considered when interpreting the findings. First, its retrospective design precludes any causal inferences. Second, important potential confounders—such as body mass index, sunlight exposure, seasonality, disease-modifying therapies, and disease duration—were not fully controlled for. Third, hematological parameters may have been influenced by intercurrent infections, which were not systematically documented. Finally, vitamin D levels were measured only once per patient, which may not accurately reflect long-term vitamin D status.

Conclusions

In this retrospective cross-sectional study, lower serum vitamin D levels were associated with higher EDSS scores and a more pronounced inflammatory hematological profile in patients with MS. These findings suggest that vitamin D status may serve as a prognostic biomarker. Careful monitoring and potential supplementation may be useful as a clinical biomarker associated with disability. Clinical trials are needed to confirm these benefits and guide recommendations.

Author Contributions:

Conceptualization, N.W.E. and M.R.Z.A.K.; methodology, N.W.E. and A.E.D.; software, S.O.; validation,

M.R.Z.A.K., K.Z., T.Z. and N.H.; formal analysis, S.O.; investigation, N.W.E., A.M. and A.B.; resources, N.W.E., N.O. and D.B.; data curation, N.W.E., N.O. and D.B.; writing–original draft preparation, N.W.E. and M.R.Z.A.K.; writing–review and editing, N.W.E., M.R.Z.A.K., N.R., S.O. and N.H.; visualization, M.R.Z.A.K., K.Z., T.Z. and N.H.; supervision, M.R.Z.A.K.; project administration, M.R.Z.A.K. All authors have read and approved the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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