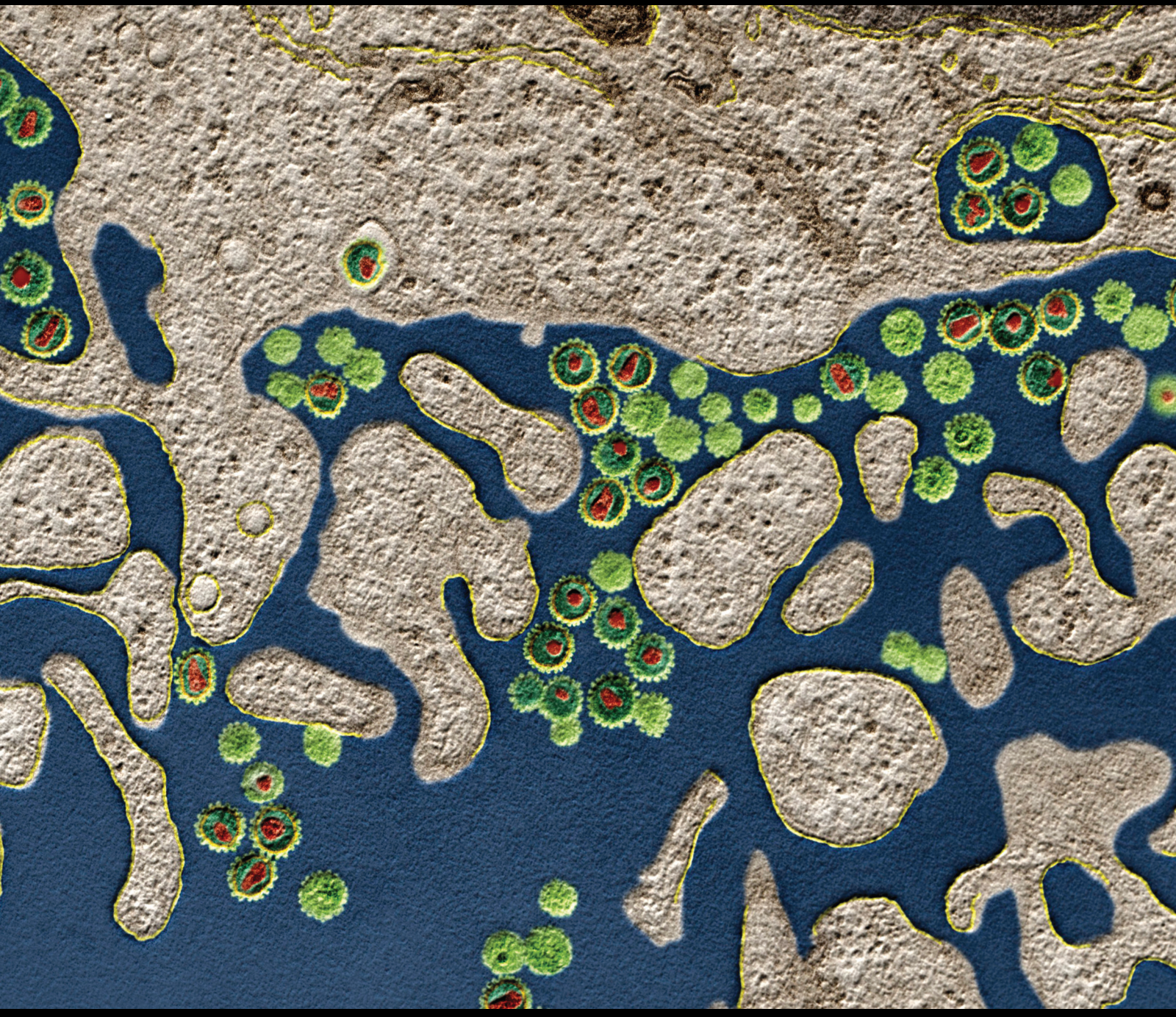
Journal of Immunology Research

The Role of Vitamin D in Chronic Disease

Lead Guest Editor: Vlad Padureanu
Guest Editors: Ana Maria Buga and Peter Malfertheiner



The Role of Vitamin D in Chronic Disease

Journal of Immunology Research

The Role of Vitamin D in Chronic Disease

Lead Guest Editor: Vlad Padureanu
Guest Editors: Ana Maria Buga and Peter Malfertheiner

Contents

Hindawi
Journal of Immunology Research
Volume 2022, Article ID 9025354, 8 pages
https://doi.org/10.1155/2022/9025354

Research Article

Vitamin D Receptor Genetic Variations May Associate with the Risk of Developing Late Fracture-Related Infection in the Chinese Han Population

Xing-qi Zhao,[1,2] **Kun Chen**,[2] **Hao-yang Wan**,[2] **Si-ying He**,[2] **Han-jun Qin**,[2] **Bin Yu**,[1,2] **and Nan Jiang**[1,2]

[1]*Division of Orthopaedics & Traumatology, Department of Orthopaedics, Southern Medical University Nanfang Hospital, Guangzhou 510515, China*
[2]*Guangdong Provincial Key Laboratory of Bone and Cartilage Regenerative Medicine, Southern Medical University Nanfang Hospital, Guangzhou 510515, China*

Correspondence should be addressed to Bin Yu; smuyubin@163.com and Nan Jiang; hnxyjn@smu.edu.cn

Received 3 August 2021; Revised 26 January 2022; Accepted 28 January 2022; Published 10 February 2022

Academic Editor: Vlad Padureanu

Variations in the vitamin D receptor (*VDR*) gene are related to several inflammatory disorders. However, the potential links between such alternations and the risk of developing late fracture-related infection (FRI) remain unclear. This study investigated associations between genetic variations in the *VDR* and susceptibility to late FRI in the Chinese Han population. Between January 2016 and December 2019, 336 patients with late FRI and 368 healthy controls were genotyped six *VDR* genetic variations, including *ApaI* (rs7975232), *BsmI* (rs1544410), *FokI* (rs2228570), *TaqI* (rs731236), *GATA* (rs4516035), and *Cdx-2* (rs11568820). Significant associations were observed between rs7975232 and FRI susceptibility in the recessive ($P = 0.019$, OR = 0.530, 95% CI 0.310–0.906) model. Patients with AA genotype had a relatively higher level of serological vitamin D (20.6 vs. 20.3 vs. 17.9 ng/ml) ($P = 0.021$) than those of AC and CC genotypes. Although no statistical differences were observed, potential correlations may exist between rs1544410 (dominant model: $P = 0.079$, OR = 0.634), rs2228570 (dominant model: $P = 0.055$, OR = 0.699), and rs4516035 (dominant model: $P = 0.065$, OR = 1.768) and the risk of FRI development. In the Chinese cohort, *ApaI* was associated with a decreased risk of developing FRI, and patients with the AA genotype had a higher vitamin D level. Further studies are required to assess the role of genetic variations in *BsmI*, *FokI*, and *GATA* in the pathogenesis of late FRI.

1. Introduction

Fracture-related infection (FRI), one of the most frequent types of bone infection, refers to osseous infection with or without the surrounding soft tissue infection following trauma and/or orthopaedic surgery [1], with infections secondary to internal fixation and open fracture as the primary types. The average incidence of FRI was approximately 5%, with 1% to 2% after closed fractures, which exceeding 30% following open fractures [2]. Currently, successful management of FRI poses substantial challenges for orthopaedic surgeons, which is primarily attributed to its characteristics of high heterogeneity, i.e., despite the same disorder, clinical efficacy varies among patients, and is affected by factors such as infection site and duration, pathogen type and virulence, immune status, and treatment strategy. Nonetheless, the overall efficacy remains unsatisfactory, posing great pressures on the patients, not only physically but also psychologically [3, 4] and economically [5, 6]. Even in patients with long-term skin ulcers and sinus drainage, malignant transformation of squamous cell carcinoma is not rare [7]. Therefore, reducing the incidence of FRI is of great personal and social significance and needs to be built on a comprehensive understanding of FRI pathogenesis.

The pathogenesis of FRI is complex, which associates with both extrinsic and intrinsic factors. However, most

previous studies have reported FRI pathogenesis from the perspective of environmental factors, which are largely controllable. Recently, growing evidence has shown that genetic predisposition also plays an important role in FRI development, with single nucleotide variation (SNV) as a representative. Several SNV sites have been found to be associated with the risk of FRI development, such as rs689466 (cyclooxygenase-2, *COX-2* gene) [8]; rs16944, rs2234663, rs1143627, rs4251961, and rs1800796 (interleukin, *IL* genes) [1, 9]; and rs2430561 (interferon-γ, *IFN-γ* gene), demonstrating that as a host factor, SNV is also involved in developing FRI.

Vitamin D participates in several biological processes, such as bone metabolism, regulation of cell proliferation and differentiation, and modulation of the immune response. Vitamin D mediates its function by binding to the vitamin D receptor (VDR), encoded by the *VDR* gene, and controls the synthesis of different proteins [10]. The *VDR* gene is highly polymorphic, significantly influencing the functioning of the VDR protein and thus may influence the occurrence of disorders. The most frequently reported *VDR* genetic variations include *ApaI* (rs7975232), *BsmI* (rs1544410), *FokI* (rs2228570), *TaqI* (rs731236), *GATA* (rs4516035), and *Cdx-2* (rs11568820). Previous studies have indicated that these SNVs are associated with the risk of developing several inflammatory disorders, such as tuberculosis [11], chronic periodontitis [12], paediatric urinary tract infection [13], and *Helicobacter pylori* infection [14]. A previous study found that *TaqI* (rs731236) and *FokI* (rs2228570) increased the risk of developing chronic osteomyelitis (COM) in the Chinese population [15]. However, both FRI and non-FRI patients were included as an entire entity for analysis, resulting in heterogeneity. Additionally, the sample size of the participants was limited. Moreover, potential relationships between different genotypes and serum vitamin D levels were not explored. Thus, the effects of genotype on vitamin D levels in patients with FRI remain unclear.

To address the above-mentioned drawbacks and questions, we investigated potential associations between *VDR* gene variations (*ApaI*, *BsmI*, *FokI*, *TaqI*, *GATA*, and *Cdx-2*) and the risk of late extremity FRI development in the Chinese Han population.

2. Materials and Methods

2.1. Definition, Inclusion and Exclusion Criteria, and Study Registration. The present study was designed as a case-control investigation, with comparisons conducted between patients with FRI and healthy controls. Late FRI is defined as bone infection with or without surrounding soft tissue infection following open fractures or internal fixation for closed fractures, with an infection duration exceeding 10 weeks [16, 17]. FRI was established based on any of the four confirmatory criteria: wound breakdown to the bone or implant, sinus or fistula connecting the bone or implant, positive pathogen culture, and histological test outcomes [18]. Participants included in the patient group were those who had been diagnosed with FRI at the Southern Medical University Nanfang Hospital between 1 January 2016 and 31 December 2019. Patients with anther aetiologies of COM (haematogenous spread and diabetic foot infection) were receiving vitamin D supplementation and diet were excluded. Eligible participants in the control group were defined as healthy after thorough examinations at the hospital. All the included participants signed the informed consent form, and this study was conducted in accordance with the tenets of the 1964 Helsinki declaration. This study was approved by the Medical Ethics Committee of the hospital (NFEC-2019-087). The protocol of this study was registered in the Chinese Clinical Trial Registry (ChiCTR1900022186).

2.2. DNA Extraction and SNV Genotype. Ethylene diamine tetraacetic acid (EDTA) peripheral blood samples (5 ml each) were collected and stored at −80°C. Then, the genomic DNA of each sample was extracted from the peripheral blood leukocytes according to the instructions of the Flexi Gene-DNA Kit (Qiagen, Valencia, CA). Six tag SNVs of the *VDR* gene (rs7975232, rs1544410, rs2228570, rs731236, rs4516035, and rs11568820) were genotyped using the Multiplex SNaPshot system (Applied Biosystems, Foster City, USA). The forward (F), reverse (R), and extension primers used for the polymerase chain reaction (PCR) and extension reactions of the six SNVs are listed in Table 1. Detailed procedures of the SNaPshot genotyping method have been described previously [15].

2.3. Outcome Parameters. Outcome parameters included comparisons regarding mutant allele frequency, homozygous and heterozygous mutant versus homozygous wild, and the dominant and recessive models of the six *VDR* SNVs for the patients and healthy controls. In addition, clinical features of the FRI cohort were analysed. Furthermore, preoperative serum levels of white blood cell (WBC) count, percentage of polymorphonuclear leukocytes (PMN%), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), serum amyloid A (SAA), and total vitamin D among different genotypes of the *VDR* gene variation(s) with clinical significance in the patient group were compared.

2.4. Statistical Analysis. Statistical analysis was conducted using the Statistical Product and Service Solutions software (version 13.0, SPSS Inc., Chicago, IL, USA). Distributions of the continuous variables were first assessed for normality using the Kolmogorov-Smirnov test. Data were presented as mean ± standard deviation (SD) or median with interquartile range (IQR) based on data distribution. For normally distributed data, Student's t-test or one-way analysis of variance (ANOVA) was used to compare differences between two groups or among three groups. Otherwise, the Mann-Whitney or Kruskal-Wallis tests were applied. When using ANOVA, a test of homogeneity of variances was also conducted. When the assumption of the homogeneity of variances was met, the LSD method was used for post hoc multiple comparisons. Otherwise, the Welch's ANOVA and Dunnett's T3 method were applied for the whole and post hoc multiple comparisons, respectively, with statistical

Table 1: PCR primers and extension primers of the six *VDR* genetic variations.

SNVs	PCR primers	Extension primers
rs7975232	F: 5′-GGCACGGGGATAGAGAAGAA-3′	5′-CTGACTGACTGACTGACTCACAGGAGCTCTCAGCTGGGC-3′
	R: 5′-GCACGGAGAAGTCACTGGA-3′	
rs1544410	F: 5′-GTGCAGGCGATTCGTAGG-3′	5′-TGGGGCCACAGACAGGCCTGC-3′
	R: 5′-ACCATCTCTCAGGCTCCAAA-3′	
rs2228570	F: 5′-GCACTGACTCTGGCTCTGA-3′	5′-CTGACTGACTGACTGACTGACTGACTCTTGCTGTTCTTACAGGGA-3′
	R: 5′-GCCTTCACAGGTCATAGCATTG-3′	
rs731236	F: 5′-TGGTGGGATTGAGCAGTGA-3′	5′-GCAGGACGCCGCGCTGAT-3′
	R: 5′-GAAGGAGAGGCAGCGGTA-3′	
rs4516035	F: 5′-CCTCTTCTTAGAACTCACTGTGC-3′	5′-TCCTTTAGCCAGGGAAGA-3′
	R: 5′-CCTTGTCCCTCTGAGCCAT-3′	
rs11568820	F: 5′-AGAACATCTTTTGTATCAGGAACT-3′	5′-CTGACTGACTGACTGACTGACTGACTCCTGAGTAAACTAGGTCACA-3′
	R: 5′-AATGTAAGAAGCTGTAGCAATGAA-3′	

Table 2: Clinical characteristics of the FRI patients included.

Clinical characteristics	Outcomes
Top injury type (no., %)	
Traffic accident	93 (40.0%)
Falling injury	50 (21.5%)
Falling from a height	27 (11.6%)
Stab injury	21 (9.0%)
Body side distribution (no., %)	
Left	159 (47.3%)
Right	170 (50.6%)
Bilateral	7 (2.1%)
Single infection site (no., %)	268 (79.8%)
Tibia	158 (59.0%)
Femur	41 (15.3%)
Calcaneus	28 (10.4%)
Foot	8 (3.0%)
Hand	7 (2.6%)
Fibula	7 (2.6%)
Humerus	6 (2.2%)
Ulna	5 (1.9%)
Radius	4 (1.5%)
Another sites	4 (1.5%)
Positive rate of intraoperative culture (%, no.)	57.1% (132/231)
Monomicrobial infection (%, no.)	63.6% (84/132)
Top detected pathogens for monomicrobial infection (no., %)	
Staphylococcus aureus	39 (46.4%)
Pseudomonas aeruginosa	12 (14.3%)
Staphylococcus epidermidis	7 (8.3%)
Enterobacter cloacae	6 (7.1%)
Enterococcus faecalis	5 (6.0%)

significance set at an adjusted P value less than 0.017 (three different genotype groups).

The genotype distributions of the healthy controls were tested to confirm the Hardy-Weinberg equilibrium (HWE) using the chi-square test. The chi-square test or Fisher's exact test was used to compare the genotype distributions and frequencies of mutant alleles and the four genetic models, with corresponding odds ratios (ORs) and 95% confidence intervals (CIs) between the patients and healthy controls. All reported values were 2-sided with a P value of less than 0.05, which was considered statistically significant.

3. Results

3.1. Demographics and FRI Characteristics. A total of 468 COM patients (357 males and 111 females) and 368 healthy controls (268 males and 100 females) were included, with no statistical differences regarding sex ratio (3.2 vs. 2.7, $P = 0.25$) and median age [48, IQR (33, 59) years vs. 46, IQR (37, 52) years, $P = 0.08$] between the two groups. Among the 468 patients, 70 and 62 patients were categorised as having diabetic foot-related and haematogenous spread-related infection, respectively, with the remaining 336 patients identified as having FRI. The mean age of the FRI patients at diagnosis was 43.3 ± 15.6 years. The clinical characteristics of the included FRI patients are depicted in Table 2.

3.2. HWE Test Outcomes of the Healthy Controls. All the genotyped *VDR* genetic variations were in the HWE among the healthy controls ($P = 0.55$ for rs7975232; $P = 0.77$ for rs1544410; $P = 0.08$ for rs2228570; $P = 0.21$ for rs731236; $P = 0.63$ for rs4516035; and $P = 0.18$ for rs11568820), demonstrating that the participants of the control group were representative.

3.3. Associations between VDR Genetic SNVs and the Risks of Developing FRI. Although no statistical difference was observed in the genotype distribution of rs7975232 between patients and healthy controls ($P = 0.059$), significant links

Table 3: Associations between the six *VDR* genetic SNVs and susceptibilities to late FRI.

SNVs	Allele or genotype	Patients ($n = 336$)	Controls ($n = 368$)	P values	OR (95% CI)
	C	480 (71.4%)	492 (66.8%)		Ref.
	A	192 (28.6%)	244 (33.2%)	0.063	0.807 (0.643-1.012)
	CC	166 (49.4%)	167 (45.4%)		Ref.
rs7975232	AC	148 (44.0%)	158 (42.9%)	0.708	0.942 (0.691-1.285)
	AA	22 (6.6%)	43 (11.7%)	0.018	0.515 (0.295-0.898)
		Dominant (AA+AC vs. CC)		0.285	0.851 (0.633-1.144)
		Recessive (AA vs. AC+CC)		0.019	0.530 (0.310-0.906)
	C	645 (96.0%)	692 (94.0%)		Ref.
	T	27 (4.0%)	44 (6.0%)	0.093	0.658 (0.403-1.076)
	CC	310 (92.3%)	325 (88.3%)		Ref.
rs1544410	CT	25 (7.4%)	42 (11.4%)	0.073	0.624 (0.371-1.049)
	TT	1 (0.3%)	1 (0.3%)	1.000	1.048 (0.065-16.834)
		Dominant (TT+CT vs. CC)		0.079	0.634 (0.380-1.057)
		Recessive (TT vs. CT+CC)		1.000	1.096 (0.068-17.584)
	A	333 (49.6%)	331 (45.0%)		Ref.
	G	339 (50.4%)	405 (55.0%)	0.085	0.832 (0.675-1.026)
	AA	80 (23.8%)	66 (17.9%)		Ref.
rs2228570	AG	173 (51.5%)	199 (54.1%)	0.090	0.717 (0.488-1.053)
	GG	83 (24.7%)	103 (28.0%)	0.066	0.665 (0.430-1.028)
		Dominant (GG+AG vs. AA)		0.055	0.699 (0.485-1.008)
		Recessive (GG vs. AG+AA)		0.323	0.844 (0.603-1.182)
	A	647 (96.3%)	700 (95.1%)		Ref.
	G	25 (3.7%)	36 (4.9%)	0.281	0.751 (0.446-1.265)
	AA	311 (92.6%)	334 (90.8%)		Ref.
rs731236	AG	25 (7.4%)	32 (8.7%)	0.528	0.839 (0.486-1.448)
	GG	0 (0.0%)	2 (0.5%)	0.500	1.006 (0.998-1.014)
		Dominant (GG+AG vs. AA)		0.390	0.790 (0.461-1.354)
		Recessive (GG vs. AG+AA)		0.500	1.005 (0.998-1.013)
	T	644 (95.8%)	718 (97.6%)		Ref.
	C	28 (4.2%)	18 (2.4%)	0.070	1.734 (0.950-3.165)
	TT	308 (91.7%)	350 (95.1%)		Ref.
rs4516035	CT	28 (8.3%)	18 (4.9%)	0.065	1.390 (0.772-2.504)
	CC	0 (0.0%)	0 (0.0%)	N/A	N/A
		Dominant (CC+CT vs. TT)		0.065	1.768 (0.959-3.259)
		Recessive (CC vs. CT+TT)		N/A	N/A
	C	380 (56.5%)	432 (58.7%)		Ref.
	T	292 (43.5%)	304 (41.3%)	0.415	1.092 (0.884-1.349)
	CC	106 (31.5%)	133 (36.1%)		Ref.
rs11568820	CT	168 (50.0%)	166 (45.1%)	0.160	1.270 (0.910-1.772)
	TT	62 (18.5%)	69 (18.8%)	0.582	1.127 (0.735-1.729)
		Dominant (TT+CT vs. CC)		0.199	1.228 (0.898-1.680)
		Recessive (TT vs. CT+CC)		0.919	0.981 (0.670-1.434)

FRI: fracture-related infection; SNV: single nucleotide variation; OR: odds ratio, CI: confidence interval; N/A: not available.

were found between this SNV site and risk of FRI development in the recessive model (OR = 0.530, 95% CI 0.310–0.906, $P = 0.019$) and homozygous models (OR = 0.515, 95% CI 0.295–0.898, $P = 0.018$), suggesting that people with the AA genotype at this site were less susceptible to FRI (Table 3).

With respect to the other five SNV sites, although no statistical differences were detected, individuals with genotype CT of rs1544410 and GG and AG of rs2228570 might be at a lower risk, whereas people with the CT genotype of rs4516035 might be at a higher risk of developing FRI (Table 3).

3.4. Stratified Analyses Regarding Links between VDR Genetic SNVs and the Risks of FRI Development by Sex and Age. As shown in Table S1, rs7975232 may also be associated to decreased susceptibility to FRI in males and patients below 60 years, demonstrating that such groups of people with the AA genotype were in a lower risk to develop FRI. In addition to rs7975232, rs2228570 may be also related to reduced risk of FRI development in females and patients below 60 years, indicating that such groups of people with the GG/AG genotypes were less susceptible to FRI. However, no significant relationships were found between the remaining *VDR* genetic SNVs and the risks of developing FRI in this cohort.

3.5. Preoperative Serum Levels of Inflammatory Biomarkers and Total Vitamin D among Different Genotypes of the Six VDR SNV Sites among the FRI Patients. Significant differences were found in the preoperative serological level of total vitamin D ($P = 0.021$) among different genotypes of rs7975232. Outcomes of post hoc multiple comparisons revealed that FRI patients with genotype AA had a relatively higher level of total vitamin D than those with AC and CC genotypes. Although no statistical differences were observed in serum TNF-α levels among the three groups ($P = 0.068$), patients with AA genotype had relatively lower TNF-α levels than those with AC ($P = 0.022$) and CC genotypes ($P = 0.031$). No significant differences were identified in the mean levels of WBC, PMN, ESR, CRP, PCT, IL-6, or SAA among the three genotypes of rs7975232 in patients with FRI (Table 4). Comparison outcomes regarding serum levels of the 8 inflammatory biomarkers and total vitamin D among different genotypes of another five SNV sites were listed in the supplemental table (Table S2).

4. Discussion

The results of the current case-control study, comprising 704 Chinese participants, demonstrated that the *VDR* gene sequence variant *ApaI* (rs7975232) was associated with a decreased risk of late FRI development, and people with the AA genotype were less susceptible to FRI. In addition, we found that patients with AA genotype had a higher total vitamin D level than those with the AC and CC genotypes, implying vitamin D may have a protective effect against bone infection. Aside from *ApaI*, we also observed that *BsmI*, *FokI*, and *GATA* variations of *VDR* may also be correlated with the risks of developing FRI, which needs to be confirmed by further studies.

The present study shared similarities and differences with a previous study [15], which also investigated *VDR* gene variations and susceptibility to bone infection. First, this study focused on late FRI only, whereas the previous study recruited different types of COM patients, which also included those with infections following haematogenous spread and diabetic foot. Second, the sample size of this study was larger than the previous one. Third, in addition to the previously analysed six inflammatory biomarkers, ESR, PMN%, and vitamin D levels were also compared among different genotypes, which provided a direct insight into the effects of *VDR* SNV on inflammatory cytokines and vitamin D levels. Our findings are discussed using the following three aspects.

First, we found that *ApaI* (rs7975232) may be associated with a decreased risk of FRI development in this cohort, with AA genotype as a protective factor. In the stratified analyses by sex and age, we found that this SNV was linked to FRI development in males and patients under 60 years. In a previous study, we did not find any significant correlation between *ApaI* and COM, which might be related to the limited sample size and the inclusion of different types of COM in the study [15]. To evaluate the potential effects of such genetic variation, preoperative levels of eight inflammatory biomarkers or cytokines and total vitamin D were compared among the different *ApaI* genotypes. The results showed that patients with AA genotype had a relatively higher vitamin D level than those of the AC and CC genotypes, implying that an elevated vitamin D level may play important roles against FRI. One of the underlying mechanisms that resulted in the AA genotype of rs7975232 having a relatively higher vitamin D level may due to the fact that this SNV site is located at the $3'$-end of the *VDR* gene, which associates with different lengths of the polyadenylate sequence and affects the mRNA stability and, therefore, different vitamin D levels [19]. In addition, although no statistical differences were observed, FRI patients with AA genotype also had relatively lower levels of WBC, ESR, PCT, TNF-α, and SAA than those with the AC and CC genotypes. Similar to our results, a recent meta-analysis found that rs7975232 was related to a reduced risk of pulmonary tuberculosis (PTB) in the African population [20]. In addition to bacterial infection, a previous study also found that rs7975232 was linked to a decreased susceptibility to hepatitis C virus (HCV) infection in a Chinese population, and the authors also indicated that low vitamin D levels may increase the risk of HCV infection and chronicity [21], which is in accordance with the present study.

Second, we found that *BsmI* (rs1544410) and *FokI* (rs2228570) might also be correlated with decreased risk of FRI development, although no statistical differences were observed. In the subgroup analyses by sex and age, we also noted that *FokI* was linked to FRI development in females and patients under 60 years. Several studies have also found positive links between the two sites and susceptibility to infectious diseases. Based on a meta-analysis of 19 studies comprising 3,644 controls and 2,635 patients, Areeshi et al. found that rs1544410 may be a risk factor for PTB in the Asian population [22], which was supported by a subsequent meta-analysis focusing on the Iranian population [23]. In addition to PTB, Shaker et al. also found that rs1544410 could be regarded as a predictor of response to combination therapy with HCV [24]. With respect to rs2228570, a recent case-control study revealed that *FokI* increased the risk of community-acquired pneumonia (CAP) development in Indian children [25]. Additionally, this SNV site was also found to be related to an elevated risk of neonatal sepsis, with the TT genotype having a relatively lower 25-hydroxyvitamin D (25OHD) level, implying that this genetic variation may participate in sepsis via its influence on peripheral vitamin D levels. Although no statistical results

Table 4: Preoperative serological levels of inflammatory biomarkers and vitamin D among different genotypes of rs7975232 in FRI patients.

rs7975232	WBC ($\times 10^9$/l)	PMN (%)	ESR (mm/1 h)	CRP (mg/l)	PCT (ng/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	SAA (mg/l)	Vitamin D (ng/ml)
AA	6.0 (5.2, 7.6)	59.5 (53.9, 63.3)	14 (7.5, 48.5)	4.6 (1.8, 14.2)	0.037 (0.029, 0.082)	7.3 (3.7, 13.8)	8.1 (6.8, 9.3)	8.5 (5.0, 28.2)	20.6 (19.6, 24.6)
AC	7.1 (5.6, 8.3)	59.2 (50.1, 65.5)	16 (8, 33)	4.5 (1.5, 10.4)	0.045 (0.034, 0.067)	5.9 (3.5, 10.7)	9.3 (7.6, 11.8)	10.7 (6.4, 31.4)	20.3 (15.0, 24.0)
CC	6.9 (5.8, 8.4)	60.0 (51.8, 66.9)	15 (7.0, 36.8)	3.9 (1.4, 13.1)	0.043 (0.027, 0.068)	6.1 (3.4, 12.7)	9.4 (7.6, 11.9)	10.8 (6.2, 19.1)	17.9 (13.5, 22.9)
P values*	0.257	0.779	0.639	0.933	0.745	0.833	0.068	0.632	0.021
Post hoc multiple comparisons#									
AA vs. AC	0.110	0.981	0.863	0.715	0.897	0.563	0.022	0.330	0.286
AA vs. CC	0.113	0.698	0.561	0.737	0.654	0.557	0.031	0.403	0.019
AC vs. CC	0.923	0.505	0.390	0.925	0.471	0.963	0.969	0.826	0.035

FRI: fracture-related infection; WBC: white blood cell count; PMN%: percentage of polymorphonuclear; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; PCT: procalcitonin; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; SAA: serum amyloid A. *These *P* values were obtained by the Kruskal-Wallis H test. #The alpha level was 0.017 for the post hoc multiple comparisons.

were obtained in our study, there was a tendency for both SNVs to be associated with decreased risks of FRI development, with the CT genotype of rs1544410 and the GG and AG genotypes of rs2228570 as underlying protective factors. Therefore, to obtain more accurate conclusions, future studies with larger sample sizes are necessary.

Third, we found that *GATA* (rs4516035) might increase susceptibility to FRI, although no statistical difference was obtained, with the CT genotype as a possible risk factor. In contrast to the previous four *VDR* genetic variations, the number of studies focusing on the potential relationship between this SNV site and the development of infectious disorders remains limited. Nonetheless, this site was found to be positively linked to muscle strength [26] and type 1 diabetes in children [27], the latter of which was achieved via its influence on serum 25OHD concentration during pregnancy in mothers. Regarding *Cdx-2* (rs11568820), we did not find any positive association between this site and FRI development, and it should be noted that the results of SNV studies are influenced by several factors apart from the sample size, such as ethnicity, selection of controls, diagnostic criteria, and detection method. Therefore, care should be taken when looking at the outcomes from a single report, and definite conclusions should be drawn on studies with a high evidence level, such as high-quality meta-analyses and systematic reviews.

Although in the past few decades, several investigations have reported relationships between *VDR* genetic variations and the risk of developing inflammatory disorders, limitations still exist. First, the sample size of most studies was limited, which directly affected the reliability of the outcomes. Second, the number of studies reporting the effects of *VDR* SNVs on serum vitamin D levels remains limited. Although our study revealed that patients with genotype AA of rs7975232 had a relatively higher vitamin D level than AC and CC genotypes, the median levels of serological vitamin D of all the three groups showed insufficient. In addition, as vitamin D levels of the healthy controls were not detected here and, also, FRI is a multifactorial-related disorder, definite conclusion cannot be made that FRI development is related or not related to vitamin D level based on the present results. Third, the detailed mechanisms of *VDR* genetic alterations in the pathogenesis of inflammation or infection remain largely unknown.

The present study had some limitations. First, although the sample size of this study was larger than that of most previous studies on FRI, it was still limited for an SNV investigation. Thus, more participants should be included in order to achieve more reliable outcomes, particularly for the *BsmI*, *FokI*, and *GATA* SNV sites. Second, although we analysed preoperative levels of the inflammatory biomarkers and vitamin D, potential influences of confounding factors such as previous interventions, seasonal influence, and diet cannot be neglected. Although we explored the potential effects of *ApaI* SNV on serological levels of different biomarkers, it is still far from sufficient, and further investigation should also focus more on serological indicators apart from vitamin D, such as calcium, phosphate, and alkaline phosphatase levels. Also, in-depth research is required to uncover potential mechanisms in detail. Third, as FRI is a highly heterogeneous disorder, the occurrence of which is associated with complex interactions between external and internal factors, our study only analysed its pathogenesis from the perspective of genetic predisposition, which created a bias. Another important factors, such as cigarette smoking and alcohol consumption, cannot be ignored, either. Nonetheless, it is still valuable as it certifies that genetic predisposition is also involved in the development of late FRI.

5. Conclusions

In summary, in this Chinese cohort, *ApaI* was found to be associated with a decreased risk of developing late FRI, with the AA genotype as a protective factor. Patients with the AA genotype at *ApaI* had a relatively higher level of vitamin D than AC and CC genotypes. Although there were tendencies regarding *BsmI*, *FokI*, and *GATA* variations and FRI development, there is still a lack of statistical support. The underlying mechanisms of *VDR* SNVs in FRI pathogenesis should be further explored.

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to the respect and protection of privacy of the patients but are available from the corresponding authors on reasonable request.

Conflicts of Interest

The authors report no conflict of interest.

Authors' Contributions

Zhao XQ, Chen K, and Wan HY contributed equally to this study. Jiang N and Yu B designed the study. Zhao XQ, Chen K, Wan HY, and He SY conducted the experiment. Jiang N and Qin HJ performed the statistical analyses. Zhao XQ, Chen K, Wan HY, and He SY participated in the sample collections. Zhao XQ and Jiang N drafted the manuscript. Jiang N and Yu B contributed to the manuscript revision. All authors approved the final submitted version.

Acknowledgments

The authors would like to thank the funding supports from the National Natural Science Foundation of China [grant no. 81802182], President Foundation of Southern Medical University Nanfang Hospital [grant no. 2020C027], and Postdoctoral Science Foundation of China [grant no. 2021M701635].

Supplementary Materials

Supplementary 1. Table S1: stratified analyses regarding associations between the six *VDR* genetic SNVs and susceptibilities to late FRI by sex and age.

Supplementary 2. Table S2: preoperative serological levels of inflammatory biomarkers and vitamin D among different genotypes of rs1544410, rs2228570, rs731236, rs4516035, and rs11568820 in the FRI patients included.

References

[1] N. Jiang, S. Y. Li, Y. F. Ma, Y. J. Hu, Q. R. Lin, and B. Yu, "Associations between interleukin gene polymorphisms and risks of developing extremity posttraumatic osteomyelitis in Chinese Han population," *Mediators of Inflammation*, vol. 2020, 9 pages, 2020.

[2] A. Trampuz and W. Zimmerli, "Diagnosis and treatment of infections associated with fracture-fixation devices," *Injury*, vol. 37, no. 2, pp. S59–S66, 2006.

[3] C. H. Tseng, W. S. Huang, C. H. Muo, Y. J. Chang, and C. H. Kao, "Increased depression risk among patients with chronic osteomyelitis," *Journal of Psychosomatic Research*, vol. 77, no. 6, pp. 535–540, 2014.

[4] C. H. Hung, J. Y. Ko, P. S. Liao et al., "Epidemiology of fatal/non-fatal suicide among patients with chronic osteomyelitis (COM): a nationwide population-based study," *Journal of International Medical Research*, vol. 48, no. 6, p. 030006052091923, 2020.

[5] N. Jiang, H. T. Wu, Q. R. Lin, Y. J. Hu, and B. Yu, "Health care costs of post-traumatic osteomyelitis in China: current situation and influencing factors," *The Journal of Surgical Research*, vol. 247, pp. 356–363, 2020.

[6] B. Parker, S. Petrou, J. P. M. Masters, F. Achana, and M. L. Costa, "Economic outcomes associated with deep surgical site infection in patients with an open fracture of the lower limb," *Bone Joint J*, vol. 100-B, no. 11, pp. 1506–1510, 2018.

[7] N. Jiang, S. Y. Li, P. Zhang, and B. Yu, "Clinical characteristics, treatment, and prognosis of squamous cell carcinoma arising from extremity chronic osteomyelitis: a synthesis analysis of one hundred and seventy six reported cases," *International Orthopaedics*, vol. 44, no. 11, pp. 2457–2471, 2020.

[8] L. Wang, N. Jiang, Q. R. Lin, C. H. Qin, Y. J. Hu, and B. Yu, "Cyclooxygenase-2 (COX-2) polymorphism rs689466 may contribute to the increased susceptibility to post-traumatic osteomyelitis in Chinese population," *Infectious Diseases*, vol. 49, no. 11-12, pp. 817–823, 2017.

[9] C. Alves De Souza, A. Queiroz Alves De Souza, Q. A. D. S. MDS, J. A. Dias Leite, M. Silva De Morais, and S. H. Barem Rabenhorst, "A link between osteomyelitis and IL1RN and IL1B polymorphisms—a study in patients from Northeast Brazil," *Acta Orthopaedica*, vol. 88, no. 5, pp. 556–561, 2017.

[10] M. Abouzid, M. Karazniewicz-Lada, and F. Glowka, "Genetic determinants of vitamin D-related disorders; focus on vitamin D receptor," *Current Drug Metabolism*, vol. 19, no. 12, pp. 1042–1052, 2018.

[11] U. Yadav, P. Kumar, and V. Rai, "FokI polymorphism of the vitamin D receptor (VDR) gene and susceptibility to tuberculosis: evidence through a meta-analysis," *Infection, Genetics and Evolution*, vol. 92, article 104871, 2021.

[12] R. C. M. Pinho, R. Dias, F. Bandeira et al., "Polymorphisms of the vitamin D receptor gene (FOKI,CDX2, andGATA) and susceptibility to chronic periodontitis in diabetic and non-diabetic individuals: a case-control study," *Journal of Investigative and Clinical Dentistry*, vol. 10, no. 1, article e12370, 2019.

[13] A. Mahyar, P. Ayazi, A. Sarkhosh Afshar et al., "Vitamin D receptor gene (FokI, TaqI, BsmI, and ApaI) polymorphisms in children with urinary tract infection," *Pediatric Research*, vol. 84, no. 4, pp. 527–532, 2018.

[14] D. J. Martins, G. C. Matos, R. S. Loiola, V. D'Annibale, and T. Corvelo, "Relationship of vitamin D receptor gene polymorphisms in Helicobacter pylori gastric patients," *Clinical and Experimental Gastroenterology*, vol. Volume 11, pp. 19–27, 2018.

[15] N. Jiang, X. Q. Zhao, C. H. Qin et al., "Association of vitamin D receptor gene TaqI, BsmI, FokI and ApaI polymorphisms and susceptibility to extremity chronic osteomyelitis in Chinese population," *Injury*, vol. 47, no. 8, pp. 1655–1660, 2016.

[16] N. Jiang, B. W. Wang, Y. M. Chai et al., "Chinese expert consensus on diagnosis and treatment of infection after fracture fixation," *Injury*, vol. 50, no. 11, pp. 1952–1958, 2019.

[17] W. J. Metsemakers, R. Kuehl, T. F. Moriarty et al., "Infection after fracture fixation: current surgical and microbiological concepts," *Injury*, vol. 49, no. 3, pp. 511–522, 2018.

[18] M. McNally, G. Govaert, M. Dudareva, M. Morgenstern, and W. J. Metsemakers, "Definition and diagnosis of fracture-related infection," *EFORT Open Rev*, vol. 5, no. 10, pp. 614–619, 2020.

[19] Y. Fang, J. B. van Meurs, A. d'Alesio et al., "Promoter and 3′-untranslated-region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: the Rotterdam study," *American Journal of Human Genetics*, vol. 77, no. 5, pp. 807–823, 2005.

[20] M. Y. Areeshi, R. K. Mandal, M. Wahid et al., "Vitamin D receptor ApaI (rs7975232) polymorphism confers decreased risk of pulmonary tuberculosis in overall and African population, but not in Asians: evidence from a meta-analysis," *Annals of Clinical and Laboratory Science*, vol. 47, no. 5, pp. 628–637, 2017.

[21] M. Wu, M. Yue, P. Huang et al., "Vitamin D level and vitamin D receptor genetic variations contribute to HCV infection susceptibility and chronicity in a Chinese population," *Infection, Genetics and Evolution*, vol. 41, pp. 146–152, 2016.

[22] M. Y. Areeshi, R. K. Mandal, S. A. Dar et al., "A reappraised meta-analysis of the genetic association between vitamin D receptor BsmI (rs1544410) polymorphism and pulmonary tuberculosis risk," *Bioscience Reports*, vol. 37, no. 3, 2017.

[23] A. Mohammadi, H. Khanbabaei, R. Nasiri-Kalmarzi, F. Khademi, M. Jafari, and N. Tajik, "Vitamin D receptor ApaI (rs7975232), BsmI (rs1544410), Fok1 (rs2228570), and TaqI (rs731236) gene polymorphisms and susceptibility to pulmonary tuberculosis in an Iranian population: a systematic review and meta-analysis," *Journal of Microbiology, Immunology, and Infection*, vol. 53, no. 6, pp. 827–835, 2020.

[24] O. Shaker, Y. Nassar, S. Ayoub, M. Elrazki, and A. Zahra, "Impact of FokI (rs10735810) and BsmI (rs1544410) on treatment of chronic HCV patients with genotype 4," *Journal of Clinical Laboratory Analysis*, vol. 30, no. 6, pp. 1021–1027, 2016.

[25] N. Awasthi, S. Awasthi, and S. Pandey, "Role of VDR gene polymorphisms with community acquired pneumonia in north Indian children: a case-control study," *International Journal of Molecular Epidemiology and Genetics*, vol. 12, no. 1, pp. 1–8, 2021.

[26] A. Bozsodi, S. Boja, A. Szilagyi, A. Somhegyi, P. P. Varga, and A. Lazary, "Muscle strength is associated with vitamin D receptor gene variants," *Journal of Orthopaedic Research*, vol. 34, no. 11, pp. 2031–2037, 2016.

[27] M. E. Miettinen, M. C. Smart, L. Kinnunen et al., "Genetic determinants of serum 25-hydroxyvitamin D concentration during pregnancy and type 1 diabetes in the child," *PLoS One*, vol. 12, no. 10, article e0184942, 2017.

Hindawi
Journal of Immunology Research
Volume 2021, Article ID 2808613, 16 pages
https://doi.org/10.1155/2021/2808613

Research Article

Association of High Calcitriol Serum Levels and Its Hydroxylation Efficiency Ratio with Disease Risk in SLE Patients with Vitamin D Deficiency

Mónica R. Meza-Meza,[1,2] José Francisco Muñoz-Valle,[3] Adolfo I. Ruiz-Ballesteros,[1,2] Barbara Vizmanos-Lamotte,[1,2] Isela Parra-Rojas,[1,4] Erika Martínez-López,[2] Edith Oregon-Romero,[3] Yolanda Fabiola Márquez-Sandoval,[2] Sergio Cerpa-Cruz,[5] and Ulises de la Cruz-Mosso[1,2]

[1]*Proyecto Inmunonutrición y Genómica Nutricional en las Enfermedades Autoinmunes, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco 44340, Mexico*
[2]*Instituto de Nutrigenética y Nutrigenómica Traslacional, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco 44340, Mexico*
[3]*Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco 44340, Mexico*
[4]*Laboratorio de Investigación en Obesidad y Diabetes, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, Chilpancingo de los Bravo, Guerrero 39087, Mexico*
[5]*Departamento de Reumatología, O.P.D. Hospital Civil de Guadalajara Fray Antonio Alcalde, Guadalajara, Jalisco 44280, Mexico*

Correspondence should be addressed to Ulises de la Cruz-Mosso; ulises_cdm@hotmail.com

Received 7 August 2021; Accepted 7 December 2021; Published 31 December 2021

Academic Editor: Vlad Padureanu

Vitamin D (calcidiol) deficiency in systemic lupus erythematosus (SLE) is more frequent than in healthy subjects (HS); it is associated with clinical activity and damage in SLE. Although calcidiol is considered the best indicator of the vitamin D serum status, its deficiency could not reflect its hydroxylation efficiency ratio and calcitriol serum status. This study was aimed at assessing the association of calcidiol and calcitriol serum levels and its hydroxylation efficiency ratio with the risk to clinical and renal disease activities in SLE patients. A cross-sectional study was conducted in 308 SLE and HS women; calcidiol and calcitriol serum levels were evaluated by immunoassays. SLE patients showed lower calcidiol serum levels *vs.* HS (21.2 *vs.* 24.2 ng/mL; $p < 0.001$). Active SLE patients presented higher calcidiol/calcitriol ratio scores *vs.* inactive SLE patients (2.78 *vs.* 1.92 pg/ng; $p = 0.02$), and SLE patients with renal disease activity showed a pattern of calcidiol-deficient levels (19.5 *vs.* 25.3 ng/mL; $p < 0.04$) with higher calcitriol levels (47 pg/mL *vs.* 41.5 pg/mL; $p = 0.02$) and calcidiol/calcitriol ratio scores (2.13 *vs.* 1.54 pg/ng; $p < 0.02$) compared to SLE patients without renal disease activity. Calcidiol levels were negatively correlated with calcitriol levels ($r = -0.26$; $p = 0.001$) and urine proteins (mg/dL) ($r = -0.39$; $p < 0.01$). Regarding calcitriol levels, it was positively correlated with the blood lymphocyte count ($r = 0.30$; $p < 0.001$) and negatively correlated with the glomerular filtration rate ($r = -0.28$; $p = 0.001$). Moreover, the calcitriol/calcidiol ratio was positively correlated with urine proteins ($r = 0.38$; $p < 0.01$). The calcidiol deficiency (OR = 2.27; 95% CI = 1.15-4.49; $p < 0.01$), high calcitriol levels ($T3^{rd}$, OR = 4.19, 95% CI = 2.23-7.90; $p < 0.001$), and a high calcitriol/calcidiol ratio score ($T3^{rd}$, OR = 5.93, 95% CI: 3.08-11.5; $p < 0.001$) were associated with the risk for SLE. In conclusion, a pattern of calcidiol deficiency with high calcitriol serum levels and a high vitamin D hydroxylation efficiency ratio was associated with disease risk in SLE patients.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by tolerance loss to nuclear self-antigens, autoantibody production, damage organs, and vitamin D deficiency [1–3]. The substantial reduction in clearance activity and formation of immune complexes may lead to local inflammation and damage with an aberrant expression of autoreactive T helper (Th) cells and proinflammatory cytokines, which are critical factors associated with SLE pathogenicity and lupus nephritis (LN) development [4, 5].

Several studies suggest that SLE is a Th type 2 (Th2) profile-driven disease. However, in SLE, different lymphocyte subsets drives its pathophysiology. The proinflammatory Th1, Th2, and Th17 profiles positively correlated with high clinical disease activity and were described as elevated in humans and murine models [4, 6]. The contributions of Th2 response to renal disease activity and LN development include the IL-6 and IL-4 production by activated basophils, which leads to autoantibody deposition in the kidney, increasing in a positive feedback loop the Th2 response, and B cell activation [4].

Currently, it has been described that the nutrients could play a crucial role in the survival, proliferation, and activation of immune cells. Vitamin D is a widely studied candidate nutrient in pathologies with an immune component [7, 8]. It can be obtained from the endogenous synthesis in the epidermis by exposure to UVB light and from foods and supplements in the form of ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) [9].

The main metabolite used to measure the vitamin D serum status is calcidiol [25 (OH)D]. However, its active metabolite calcitriol [1α,25$(OH)_2$D] exerts its effects through genomic mechanisms modulated by the nuclear vitamin D receptor (VDR)/retinoid X receptor (RXR) complex to bind to vitamin D response elements (VDRE) in target genes of several immune cells [9]. Hence, calcitriol is considered an immunomodulator that participates in the control of self-tolerance and influences immune cell differentiation and production of several cytokines [1, 8, 10].

Calcitriol suppresses the differentiation of Th1 cells while promoting the formation of tolerogenic dendritic cells, T regulatory cells (Tregs), Th2 differentiation, IL-4 production, the anti-inflammatory status, and reduction of autoantibodies [7, 11, 12]. Nevertheless, the effects of calcitriol on B cells that are pivotal in SLE are still unclear because it depends on its available quantity, cytokine microenvironment, activation, and differentiation status of B cells [11].

In a biphasic dose-dependent manner, high calcitriol concentrations stimulate the plasma cell development; when B cells are terminally differentiating, induce a high IL-10 production, B cell hyperactivity, and autoantibody production associated with high clinical disease activity in SLE [5, 11, 13], highlighting that vitamin D could be an immunomodulatory nutrient that plays a bimodal role during physiological and pathological events.

Notably, in SLE, vitamin D deficiency has been associated with high severity, progression, and comorbidity development such as LN [14–16]. Its deficiency in SLE patients is more frequent compared to healthy individuals [14, 17], with a prevalence rate of calcidiol insufficiency (<30 ng/mL) of 38–96% and deficiency (<20 ng/mL) of 3–35% in several populations [1, 7, 14–16, 18, 19].

Most studies in SLE patients have focused on evaluating the calcidiol serum levels with controversial findings of its relationship with the clinical and renal disease activities or comorbidities, and the association of calcidiol with the active metabolite calcitriol has not been previously reported in other studies conducted in SLE patients so far.

Therefore, the physiological complexity of the synthetic pathway of vitamin D suggests that the efficient regulation of vitamin D hydroxylation might be more crucial than the concentration of any D metabolite alone, which could be altered in SLE. Although calcidiol is considered the best indicator of the serum vitamin D status, its deficiency could not reflect calcitriol's serum status. According to this, the calcitriol/calcidiol ratio assessment, which theoretically represents how many picograms of calcitriol are produced per nanogram of circulating calcidiol, could be considered representative of the vitamin D hydroxylation efficiency [20]. Based on these previous findings, the aim of our study was at assessing the association of calcidiol, calcitriol, and its hydroxylation efficiency ratio with the risk to clinical and renal disease activities in SLE patients.

2. Material and Methods

2.1. Participants. A cross-sectional study was conducted in 308 women from an unrelated Mexican-mestizo population, made up of two groups: the first group was made up of 157 SLE women patients classified according to the 1997 SLE American College of Rheumatology (ACR) [21], recruited between 2017 and 2020 from the Rheumatology Department of the *Hospital Civil Fray Antonio Alcalde*, Guadalajara, Jalisco, Mexico. SLE patients had no recent infections, trauma, surgery, pregnancy, or systemic autoimmune conditions unrelated to SLE.

The second group was composed of 151 healthy female subjects (HS) as a reference group, recruited from the same geographical area. The HS included did not refer to recent infections, trauma, surgery, pregnancy, or autoimmune conditions; they were also asked about the presence of autoimmune diseases in their families. They did not mention that their close relatives like siblings, parents, and grandparents presented autoimmune diseases. Both study groups presented an ancestry of at least back three generations in the same geographical region.

2.2. Ethical Considerations. Before enrollment in the study, all participants provided signed written informed consent. The Research Ethical Committee of the *Centro Universitario de Ciencias de la Salud*, *Universidad de Guadalajara* (CI-03419 CUCS-UdG), and the *Hospital Civil Fray Antonio Alcalde* (no. 280/19) approved the research study, according to the international ethical guidelines.

2.3. SLE Clinical Disease Activity and Damage Indexes. The clinical disease activity presented at enrollment in the study was evaluated by the Mexican Systemic Lupus Erythematosus Disease Activity Index (Mex-SLEDAI), which is a validated clinical disease activity index adjusted for the Mexican-mestizo population [22], and the disease damage was evaluated according to the Systemic Lupus International Collaborating Clinics-American College of Rheumatology Damage Index (SLICC-ACR DI) criteria [23].

2.4. Quantification of Vitamin D Metabolites. A blood sample was obtained from each patient from antecubital venipuncture, collected in the morning after an overnight fast (12 h), and then, centrifuged for 10 min to obtain the serum. Calcidiol and calcitriol serum levels were determined using an ELISA plate reader (Multiskan GO, Thermo Scientific™ 51119000, USA) with commercial competitive ELISA assays, according to the manufacturer's instructions. For the quantification of calcidiol (25-hydroxy-vitamin D), the human soluble 25-OH Vitamin D ELISA Kit (detection limit of 1.6 ng/mL, Eagle Biosciences®, VID31-K01, USA) was used and the calcitriol serum quantification (1,25α-dihydroxyvitamin D_3) was by the human soluble 1,25$\alpha(OH)_2D_3$ ELISA kit (sensitivity < 0.10 pg/mL, Cusabio®, CSB-E0512h, China).

2.5. Classification Criteria and Definitions. The reference cutoff values for the interpretation of serum calcidiol levels were (a) deficiency (<20 ng/mL), (b) insufficiency (≥20 to <30 ng/mL), and (c) sufficiency (≥30 ng/mL), according to cutoff values reported [24, 25].

For calcitriol, the reference cutoff values were 15–60 pg/mL [26, 27]. However, for the evaluated sample, we stratified the calcitriol levels classified into tertiles: (a) low calcitriol serum levels = $T1^{st}$ (0.33 to <33.6 pg/mL), (b) average calcitriol serum levels = $T2^{nd}$ (≥33.6 to <48.7 pg/mL), and (c) high calcitriol serum levels = $T3^{rd}$ (≥48.7 to 157.3 pg/mL).

To estimate the vitamin D hydroxylation efficiency, we calculated the calcitriol/calcidiol ratio based on values of calcitriol (pg/mL) and calcidiol (ng/mL), which resulted in arbitrary units (pg/ng) that should indicate how many pg of calcitriol is produced per ng of circulating calcidiol [20]. The calcitriol/calcidiol ratio score was also stratified into tertiles: (a) low conversion rate of calcidiol to calcitriol = $T1^{st}$ (0.01 to <1.36 pg/ng), (b) average conversion rate of calcidiol to calcitriol = $T2^{nd}$ (≥1.36 to ≤2.23 pg/ng), and (c) high conversion rate of calcidiol to calcitriol = $T3^{rd}$ (≥2.23 to 23.6 pg/ng).

Also, the glomerular filtration rate (GFR) was estimated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on serum creatinine (mg/dL) and using the parameters sex, race, and age, expressed in mL/min/1.73 m^2 of the body surface area [28]. The GFR (mL/min/1.73m^2) was classified by the Kidney Disease: Improving Global Outcomes 2012 Clinical Practice Guideline (KDIGO 2012) categories: (a) G1 (normal or high ≥ 90), (b) G2 (mildly decreased: 60–89), (c) G3a (mildly to moderately decreased: 45-59), (d) G3b (moderately to severely decreased: 30–44), (e) G4 (severely decreased: 15–29), and (f) G5 (kidney failure < 15) [29].

2.6. Statistical Analysis. The statistical analyses were performed with the software STATA v 15 (College Station, TX, USA) and GraphPad Prism v 8.0 (San Diego, CA, USA). The statistical power was evaluated according to the calculation of the sample size, performed with an estimated error margin of 2% with a confidence degree of 95% and an expected prevalence of serum vitamin D insufficiency and deficiency excess in SLE patients of 83–96% and 3.1–4.6%, respectively, reported in a previous study in Mexican SLE patients [30].

The Shapiro–Wilk test was used to determine the nonparametric and parametric distribution of the continuous variables. For the descriptive analysis, the nominal discontinuous variables were expressed as frequencies; the continuous variables with parametric distribution were expressed as means ± standard deviation (SD) and the nonparametric variables as medians and percentiles 5th–95th. For the inferential analysis, the χ^2 test was used to compare proportions. For parametric quantitative determinations of two groups, Student's t-test was used and the Mann–Whitney U test was used for nonparametric quantitative determinations. We used linear and logistic regression models to evaluate the association and contribution of the calcidiol and calcitriol serum levels as well as the calcitriol/calcidiol ratio to the clinical and renal disease activities. The differences were considered significant with a p value < 0.05.

3. Results

3.1. Vitamin D Serum Metabolite Levels in SLE Patients and Healthy Subjects. A total of 157 female SLE patients were evaluated with a median age of 37 (20–59) years old. According to the Mex-SLEDAI score, 57% of SLE patients were without clinical disease activity (Mex-SLEDAI < 2) and 45% were with clinical disease activity (Mex-SLEDAI ≥ 2). As a reference healthy group representative of the same population, a total of 151 HS women were evaluated with a median age of 30 (19–59) years old.

SLE patients showed lower calcidiol serum levels compared to HS (SLE = 21.2 *vs.* HS = 24.2 ng/mL; $p < 0.001$), both classified as calcidiol insufficiency (20 to <30 ng/mL) (Figure 1(a)). According to the calcidiol reference values, a higher frequency of SLE patients showed calcidiol deficiency (<20 ng/mL) (SLE = 45% *vs.* HS = 27%), followed by calcidiol insufficiency (≥20 to <30 ng/mL) (SLE = 38% *vs.* HS = 49%), and low calcidiol sufficiency frequency (≥30 ng/mL) (SLE = 17% *vs.* HS = 24%) ($p < 0.01$) (data not shown).

Regarding calcitriol serum levels, SLE patients presented higher values with 46.8 pg/mL *vs.* 34.4 pg/mL in HS ($p < 0.001$) (Figure 1(b)). The calcitriol serum levels were categorized in tertiles, and we observed in the SLE patients a higher frequency in the third tertile classified as high calcitriol serum levels ($T3^{rd}$ = 43%), followed by the second tertile with the average calcitriol levels ($T2^{nd}$ = 37%), and the first tertile with the low calcitriol levels ($T1^{st}$ = 20%), compared with HS who presented a higher frequency in the first tertile ($T1^{st}$ = 47%), followed in decreasing order by the $T2^{nd}$ (30%), and the $T3^{rd}$ (24%) ($p < 0.001$) (data not shown).

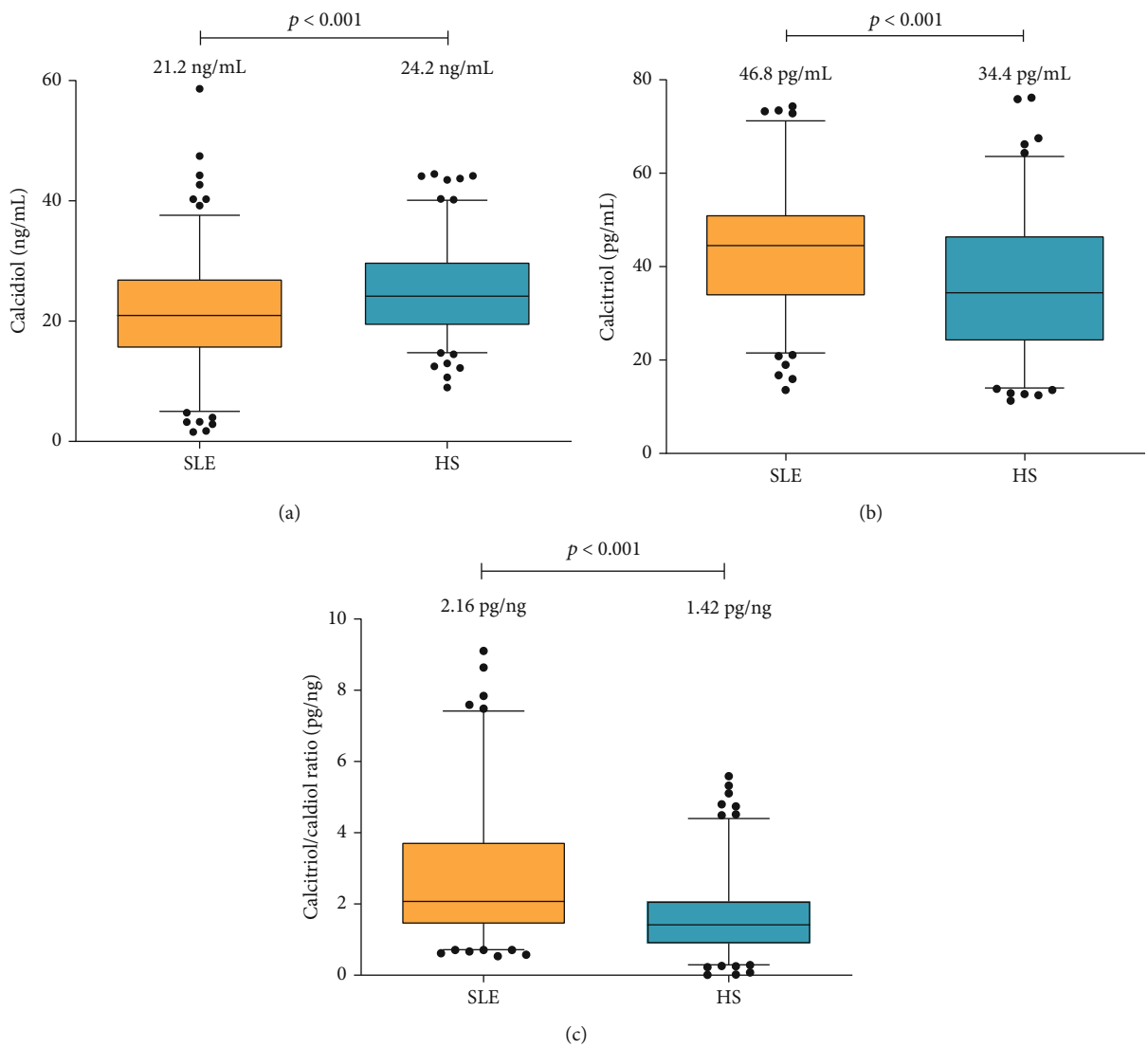


Figure 1: Vitamin D serum metabolites and vitamin D hydroxylation efficiency ratio from SLE patients *vs.* HS. (a) Calcidiol serum levels of SLE patients (5.1–40.4 ng/mL) *vs.* HS (14.8–40.2 ng/mL); (b) calcitriol serum levels of SLE patients (22.1–103 pg/mL) *vs.* HS (8.92–89.8 pg/mL); (c) efficiency of vitamin D hydroxylation by the calcitriol/calcidiol ratio of SLE patients (0.73–10.5 pg/ng) *vs.* HS (0.30–4.31 pg/ng). Data provided in median ($p5^{th}$–$p95^{th}$ in parenthesis); Mann–Whitney *U* test. SLE: systemic lupus erythematosus; HS: healthy subjects.

The vitamin D hydroxylation efficiency was estimated by the calcitriol/calcidiol ratio, and the SLE patients showed a higher score of 2.16 pg/ng, interpreted as a high-calcidiol-to-calcitriol conversion rate compared to HS (1.42 pg/ng) ($p < 0.001$) (Figure 1(c)).

3.2. Vitamin D Serum Levels Stratified by Clinical Disease Activity. To evaluate the association of calcidiol serum levels with the clinical disease activity, the SLE patients were stratified according to the Mex-SLEDAI score in inactive SLE patients (Mex-SLEDAI < 2) and active SLE patients (Mex-SLEDAI ≥ 2). Active SLE patients showed a higher frequency of renal disease activity (45%; $p = 0.01$) than inactive SLE patients (Table 1). A trend of lower calcidiol serum levels (21.2 ng/mL; $p = 0.14$) and a higher frequency of calcidiol deficiency (48%; $p = 0.34$) was observed in active SLE patients in comparison to inactive SLE patients (Table 1). No differences were observed on calcitriol serum levels between active and inactive SLE patients. However, active SLE patients showed a trend of higher frequency of calcitriol serum levels in the third tertile ($T3^{rd} = 50\%$) compared to inactive SLE patients ($p = 0.26$) (Table 1).

About the calcitriol/calcidiol ratio, the active SLE patients showed a higher calcitriol/calcidiol ratio (2.78 pg/ng; $p = 0.02$) and lower serum calcium levels (8.94 mg/dL; ($p = 0.01$) compared to inactive SLE patients (Table 1).

3.3. Vitamin D Serum Levels in SLE Patients with Renal Disease Activity. Clinical characteristics and vitamin D metabolite serum levels were evaluated in a subgroup of SLE patients according to the presence or absence of renal disease activity. About their clinical characteristics, SLE patients with renal disease activity showed higher values of the damage score (SLICC = 1; $p = 0.02$) and clinical disease

Table 1: Clinical characteristics by clinical activity in SLE patients.

Variable	Total $n = 146$	SLE patients Clinical activity (Mex-SLEDAI ≥ 2) $n = 63$	Clinical inactivity (Mex-SLEDAI < 2) $n = 83$	p value
Age (years)[a]	37 (20–58)	36 (21–56)	37 (20–60)	0.65
Disease evolution time (years)				0.44
<5 years[b]	44 (63/143)	48 (30/63)	41 (33/80)	
>5 years[b]	56 (80/143)	52 (33/63)	59 (47/80)	
SLICC ACR-DI[a]	0 (0–4)	1 (0–4)	0 (0–2)	**<0.001**
Damage (SLICC ≥ 1)[b]	42 (55/130)	64 (36/56)	26 (19/74)	**<0.001**
Renal activity [b]	30 (26/87)	45 (15/33)	20 (11/54)	**0.01**
Renal insufficiency [b]	14 (12/83)	21 (7/34)	10 (5/49)	0.18
Serum variables				
ANAs (+)[b]	81 (100/123)	81 (44/54)	81 (56/69)	0.96
Anti-dsDNA (+)[b]	42 (59/139)	70 (16/23)	53 (20/38)	0.19
C3 (mg/dL)[a]	114 (55.3–150)	101 (1.17–123)	115 (55.3–156)	0.20
C4 (mg/dL)[c]	15.5 ± 8.01	12.7 ± 7.47	17.2 ± 8.09	0.19
Calcidiol (ng/mL)[a]	21.9 (5.1–40.4)	21.2 (3.26–36.6)	22.4 (12.0–40.4)	0.14
Calcidiol reference values % (n)				0.34
Sufficiency (≥30 ng/mL)[b]	18 (26/144)	13 (8/62)	22 (18/82)	
Insufficiency (≥20 to <30 ng/mL)[b]	38 (55/144)	39 (24/62)	38 (31/82)	
Deficiency: (<20 ng/mL)[b]	44 (63/144)	48 (30/62)	40 (33/82)	
Calcitriol (pg/mL)[a]	46.9 (22.1–101)	48.5 (21.7–117)	46.2 (22.1–95.2)	0.32
Calcitriol tertiles % (n)				0.26
$T3^{rd}$ (≥48.7 to 157.3 pg/mL)[b]	43 (61/142)	50 (30/60)	38 (31/82)	
$T2^{nd}$ (≥33.6 to <48.7 pg/mL)[b]	37 (53/142)	30 (18/60)	43 (35/82)	
$T1^{st}$ (0.33 to <33.6 pg/mL)[b]	20 (28/142)	20 (12/60)	19 (16/82)	
Calcitriol/calcidiol ratio (pg/ng)[a]	2.15 (0.73–9.13)	2.78 (0.84–18)	1.92 (0.71–6.43)	**0.02**
Calcitriol/calcidiol ratio tertiles % (n)				0.58
$T3^{rd}$ (≥2.23 to 23.6 pg/ng)[b]	47 (67/141)	52 (31/60)	44 (36/81)	
$T2^{nd}$ (≥1.36 to ≤2.23 pg/ng)[b]	31 (44/141)	27 (16/60)	35 (28/81)	
$T1^{st}$ (0.01 to <1.36 pg/ng)[b]	21 (30/141)	21 (13/60)	21 (17/81)	
Serum calcium (mg/dL)[c]	9.24 ± 0.53	8.94 ± 0.61	9.44 ± 0.35	**0.01**

[a]Data provided in medians (percentile: $p05^{th}$–$p95^{th}$), Mann–Whitney test. [b]Data provided in percentages (n/total patients), χ^2 test. [c]Data provided in mean ± SD, Student t-test. The bold numbers indicate the variables with significant differences. SLICC: Systemic Lupus International Collaborating Clinics, American College of Rheumatology Damage Index; Mex-SLEDAI: Mexican Systemic Lupus Erythematosus Disease Activity Index; ANAs: antinuclear antibodies; Anti-dsDNA: anti-double-stranded DNA antibodies; C3 and C4: complement.

activity score (Mex-SLEDAI = 2; $p < 0.01$) compared to SLE without renal disease activity (Table 2). Additionally, the SLE patients with renal disease activity showed higher frequency of proteinuria (48% *vs.* 3%; $p < 0.001$), microalbuminuria (79% *vs.* 21%; $p = 0.001$), hematuria (80% *vs.* 22%; $p < 0.001$), and pyuria (90% *vs.* 61%; $p = 0.02$) in comparison with SLE patients without renal disease activity (data not shown).

Regarding the evaluation of vitamin D metabolites, SLE patients with renal disease activity showed lower calcidiol serum levels (19.5 ng/mL) classified as calcidiol deficiency in comparison to SLE patients without renal disease activity (25.3 ng/mL) classified as insufficiency ($p < 0.04$) (Figure 2(a)). Moreover, higher calcidiol deficiency was observed in SLE patients with renal disease activity (52%) compared to SLE patients without renal disease activity (25%) ($p = 0.03$) (Table 2). By contrast, SLE patients with renal disease activity showed higher calcitriol levels (47 pg/mL) ($p = 0.02$) (Figure 2(b)) and a higher frequency of high calcitriol levels in the third tertile ($T3^{rd}$: 41%) ($p = 0.03$) compared to SLE patients without renal activity (Table 2).

Table 2: Clinical characteristics by renal activity in SLE patients.

Variable	Total $n = 88$	SLE patients		p value
		Renal activity $n = 27$	No renal activity $n = 61$	
Age (years)[a]	33 (20–59)	32 (19–58)	33 (20–59)	0.44
Disease evolution time % (n)				0.06
>5 years[b]	48 (41/85)	33 (9/27)	55 (32/58)	
SLICC ACR-DI[a]	0 (0–04)	1 (0–5)	0 (0–3)	**0.02**
Damage (SLICC ≥ 1)[b]	39 (29/74)	56 (13/23)	31 (16/51)	**0.04**
Mex-SLEDAI[a]	0 (0–8)	2 (0–8)	0 (0–7)	**<0.01**
Clinical disease activity (≥2)[b]	38 (33/87)	58 (15/26)	30 (18/61)	**0.01**
Renal insufficiency[b]	14 (12/83)	37 (10/27)	4 (2/56)	**<0.001**
Serum variables				
ANAs (+)[b]	94 (63/67)	96 (24/25)	93 (39/42)	0.60
Anti-dsDNA (+)[b]	59 (36/61)	82 (18/22)	46 (18/39)	**<0.01**
C3 (mg/dL)[a]	114 (55.3–150)	75 (55.3–120)	115 (1.17–156)	**0.03**
C4 (mg/dL)[c]	15.5 ± 8.01	13.4 ± 9.37	16.3 ± 7.53	0.43
Calcidiol reference values % (n)				**0.03**
Sufficiency (≥30 ng/mL)[b]	24 (20/82)	27 (7/26)	21 (13/61)	
Insufficiency (≥20 to <30 ng/mL)[b]	43 (35/82)	23 (6/26)	52 (32/61)	
Deficiency (<20 ng/mL)[b]	33 (27/82)	50 (13/26)	26 (16/61)	
Calcitriol tertiles % (n)				**0.03**
$T3^{rd}$ (≥48.7 to 157.3 pg/mL)[b]	24 (21/88)	41 (11/27)	16 (10/61)	
$T2^{nd}$ (≥33.6 to <48.7 pg/mL)[b]	49 (43/88)	44 (12/27)	51 (31/61)	
$T1^{st}$ (0.33 to <33.6 pg/mL)[b]	27 (24/88)	15 (4/27)	33 (20/61)	
Calcitriol/calcidiol ratio tertiles % (n)				0.26
$T3^{rd}$ (≥2.23 to 23.6 pg/ng)[b]	29 (25/87)	38 (10/26)	25 (15/61)	
$T2^{nd}$ (≥1.36 to ≤2.23 pg/ng)[b]	41 (36/87)	42 (11/26)	41 (25/61)	
$T1^{st}$ (0.01 to <1.36 pg/ng)[b]	30 (26/87)	19 (5/26)	34 (21/61)	
Serum calcium (mg/dL)[c]	9.25 ± 0.53	9.1 ± 0.90	9.3 ± 0.39	0.43

[a]Data provided in medians (percentile: $p05^{th}$–$p95^{th}$), Mann–Whitney test. [b]Data provided in percentages (n/total patients), χ^2 test. [c]Data provided in mean ± SD, Student t-test. The bold letters indicate the variables with significant differences. SLICC: Systemic Lupus International Collaborating Clinics, American College of Rheumatology Damage Index; Mex-SLEDAI: Mexican Systemic Lupus Erythematosus Disease Activity Index; ANAs: antinuclear antibodies; Anti-dsDNA: anti-double-stranded DNA antibodies; C3 and C4: complement; GFR: glomerular filtration rate; G1: normal or high; G2: mildly decrease; G3a: mildly to moderately decrease; G3b: moderately to severely decrease; G4: severely decreased; G5: kidney failure.

About the calcitriol/calcidiol ratio, SLE patients with renal disease activity showed a higher conversion rate of calcidiol to calcitriol (2.13 pg/ng), compared to SLE patients without renal disease activity (1.54 pg/ng; $p < 0.02$) (Figure 2(c)).

3.4. Calcitriol Serum Levels and Vitamin D Hydroxylation Efficiency Ratio Stratified by the Calcidiol Reference Values. Based on the previous result, we evaluated the calcitriol serum levels and the calcitriol/calcidiol ratio pattern according to the calcidiol reference values stratified in deficiency, insufficiency, and sufficiency of vitamin D.

We observed significant differences with higher calcitriol serum levels in SLE patients *vs.* HS with calcidiol deficiency (SLE = 51.7 pg/mL [32.6–109] *vs.* HS = 32.7 pg/mL [7.84–64.8; $p < 0.001$), followed by the calcidiol insufficiency group (SLE = 43.9 pg/mL [21.1–97.5] *vs.* HS = 36.7 pg/mL [7.94–102]; $p < 0.01$), with similar calcitriol values in a calcidiol sufficiency condition (SLE = 44.5 pg/mL *vs.* HS = 34.9 pg/mL; $p = 0.20$). Notably, a pattern of decreasing calcitriol serum levels as calcidiol sufficiency was achieved in both study groups was observed (Figure 3(a)).

About the efficiency of vitamin D hydroxylation, SLE patients showed a higher calcidiol to calcitriol conversion compared to HS in the range of calcidiol deficiency (SLE = 4.02 pg/ng [1.78–18] *vs.* HS = 1.95 pg/ng [0.43–4.01]; $p < 0.001$), followed in descending order by the calcidiol insufficiency status (SLE = 1.79 pg/ng [0.84–4.51] *vs.* HS = 1.42 pg/ng [0.30–4.53]; $p < 0.01$), while in the calcidiol sufficiency range, SLE patients and HS showed a similar calcitriol/calcidiol ratio (SLE = 1.14 pg/ng *vs.* HS = 0.95 pg/ng; $p = 0.49$). This pattern highlights that in a serum calcidiol deficiency, the conversion rate of calcidiol to calcitriol increases and the conversion decreases as sufficiency is achieved (Figure 3(b)).

Additionally, when we compare the SLE patients according to the presence of clinical disease activity and stratified by the calcidiol reference values, we observed higher

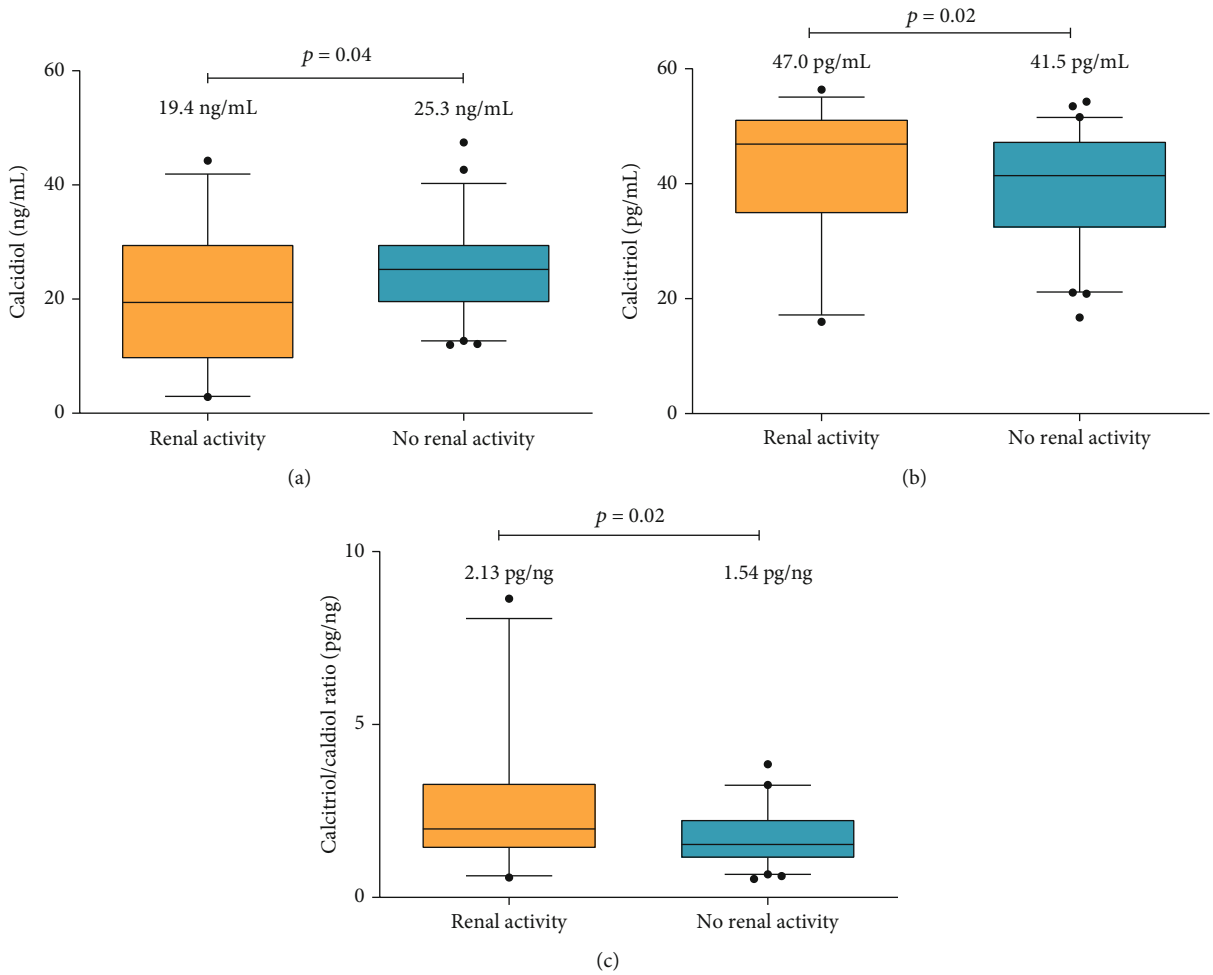


Figure 2: Vitamin D serum metabolites and vitamin D hydroxylation efficiency ratio from SLE patients stratified by renal disease activity. (a) Calcidiol serum levels (ng/mL) from SLE patients with renal activity (3.26–36.6 ng/mL) and no renal activity (12.7–40.4 ng/mL); (b) calcitriol serum levels (pg/mL) from SLE patients with renal activity (19.0–53.2 pg/mL) and no renal activity (21.8–51.2 pg/mL); (c) calcitriol/calcidiol ratio (pg/ng) from SLE patients with renal activity (0.76–15.3 pg/ng) and no renal activity (0.71–3.21 pg/ng). Data provided in median ($p5^{th}$–$p95^{th}$ in parenthesis), Mann–Whitney test. SLE: systemic lupus erythematosus.

calcitriol serum levels in active SLE patients with calcidiol deficiency (clinical disease activity = 54.9 pg/mL [23.7–131] *vs.* clinical disease inactivity = 46.5 pg/mL [23.4–98.4]; $p = 0.04$) and no differences between calcitriol were observed in the comparison to active *vs.* inactive SLE patients in the ranges of calcidiol insufficiency (clinical disease activity = 38.1 pg/mL *vs.* clinical disease inactivity = 45.4 pg/mL; $p = 0.54$), and calcidiol sufficiency (clinical disease activity = 36.2 pg/mL *vs.* clinical disease inactivity = 46.2 pg/mL; $p = 0.52$) (Figure 3(c)).

With respect to the vitamin D hydroxylation efficiency, we observed a higher calcitriol/calcidiol ratio in active SLE patients (clinical disease activity = 5.56 pg/ng [1.90–22.5] *vs.* clinical disease inactivity = 3.20 pg/ng [1.40–1.43]; $p = 0.001$), with a descending pattern of conversion of calcidiol to calcitriol with no significant differences in SLE patients with calcidiol insufficiency (clinical disease activity = 1.56 pg/ng *vs.* clinical disease inactivity = 1.80 pg/ng; $p = 0.36$) and with calcidiol sufficiency (clinical disease activity = 0.87 pg/ng *vs.* clinical disease inactivity = 1.22 pg/ng; $p = 0.39$). These results highlight that active SLE patients with calcidiol deficiency have higher calcitriol serum levels, with a high conversion rate of calcidiol to calcitriol compared to inactive SLE patients with a similar calcidiol deficiency (Figure 3(d)).

Because renal disease activity is one of the main variables contributing to the clinical disease activity, we made the same comparative analysis about the calcitriol serum levels and the vitamin D hydroxylation efficiency scores stratified by calcidiol serum reference values. We observed that SLE patients with renal disease activity and calcidiol deficiency showed higher calcitriol serum levels (renal disease activity = 48.7 pg/mL [33.3–53.2] *vs.* renal disease inactivity = 44.7 pg/mL [28.7–51.7]; $p = 0.03$) and the same pattern was observed in the range of calcidiol insufficiency (renal disease activity = 47.5 pg/mL [39.3–56.5] *vs.* renal disease inactivity = 38.1 pg/mL [19.5–52.1]; $p = 0.02$) in comparison with renal-inactive patients. This differential pattern was not observed in the comparison of renal active SLE versus renal inactive patients, both with calcidiol sufficiency (renal

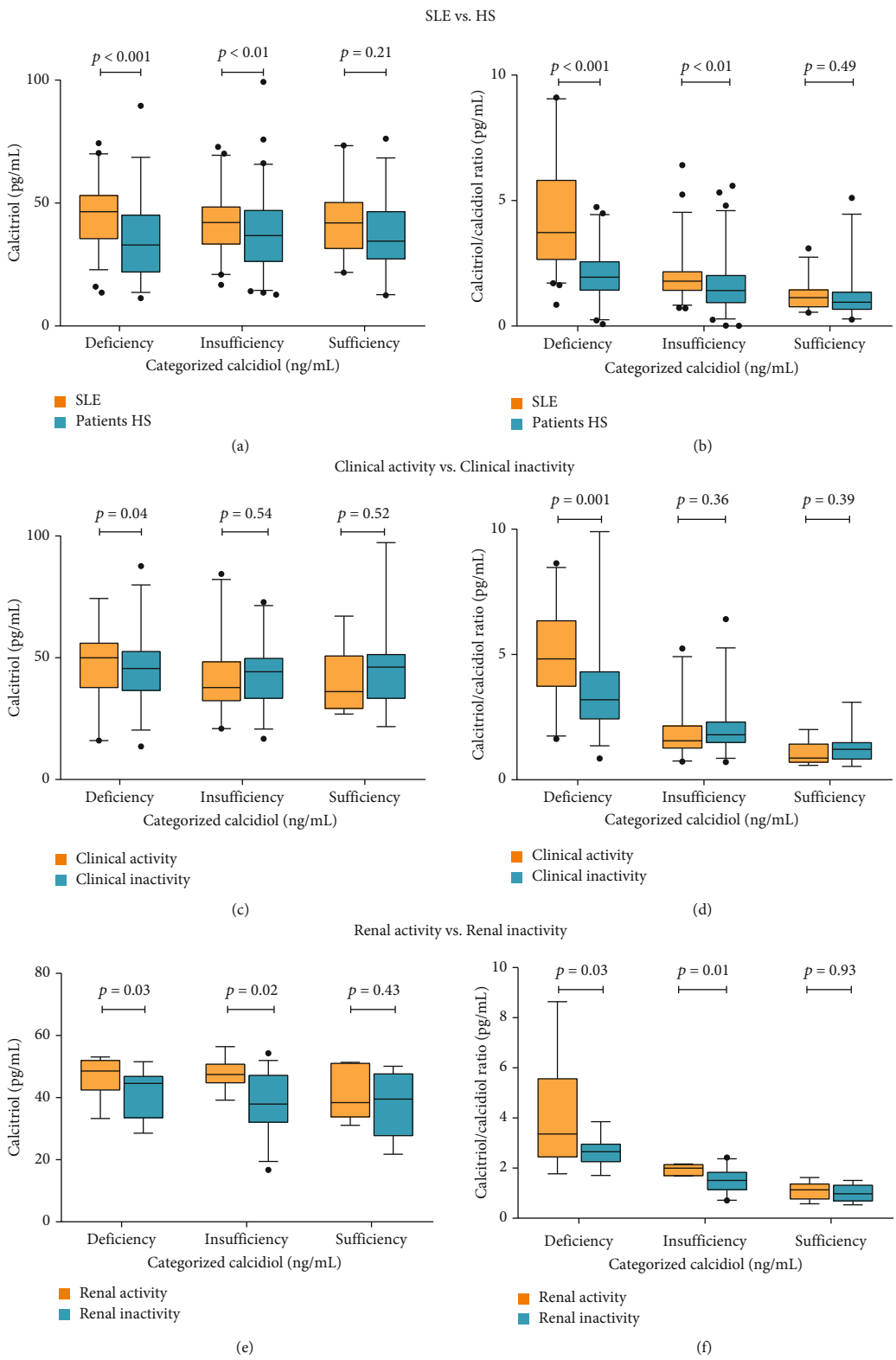


Figure 3: Calcitriol serum levels and vitamin D hydroxylation efficiency ratio stratified by the calcidiol reference values. (a) Calcitriol serum levels (pg/mL) from SLE patients *vs.* HS, (b) Efficiency of vitamin D hydroxylation (pg/ng) from SLE patients *vs.* HS; (c) calcitriol serum levels (pg/mL) from SLE patients with clinical activity *vs.* SLE patients with clinical inactivity; (d) efficiency of vitamin D hydroxylation (pg/ng) from SLE patients with clinical activity *vs.* SLE patients with clinical inactivity; (e) calcitriol serum levels (pg/mL) from SLE patients with renal activity *vs.* SLE patients with no renal activity, (f) efficiency of vitamin D hydroxylation (pg/ng) from SLE patients with renal activity *vs.* SLE patients with no renal activity. Data provided in median, Mann–Whitney test. Calcidiol reference values: deficiency (<20 ng/mL), insufficiency (≥20 to <30 ng/mL), and sufficiency (≥30 ng/mL).

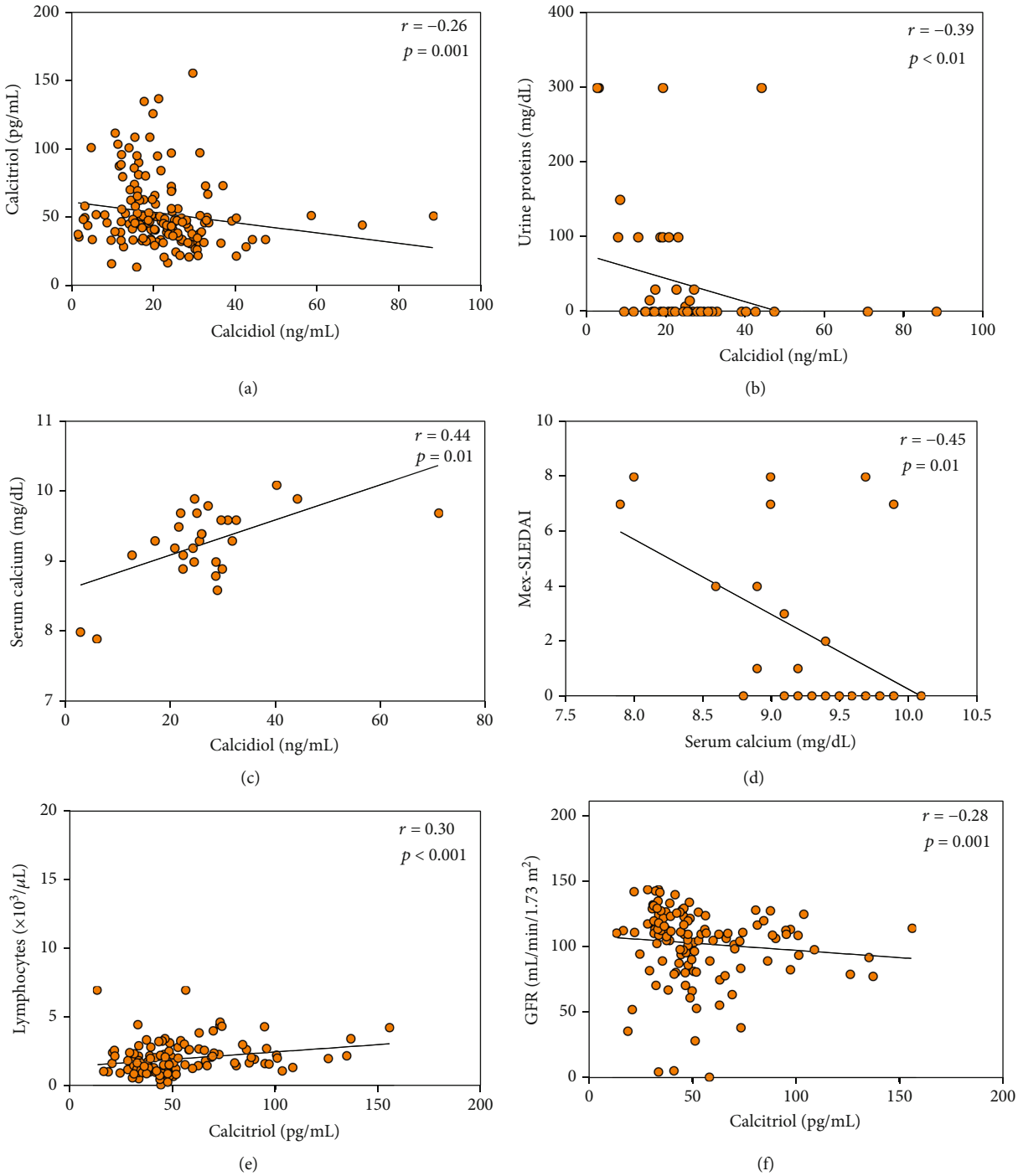


Figure 4: Continued.

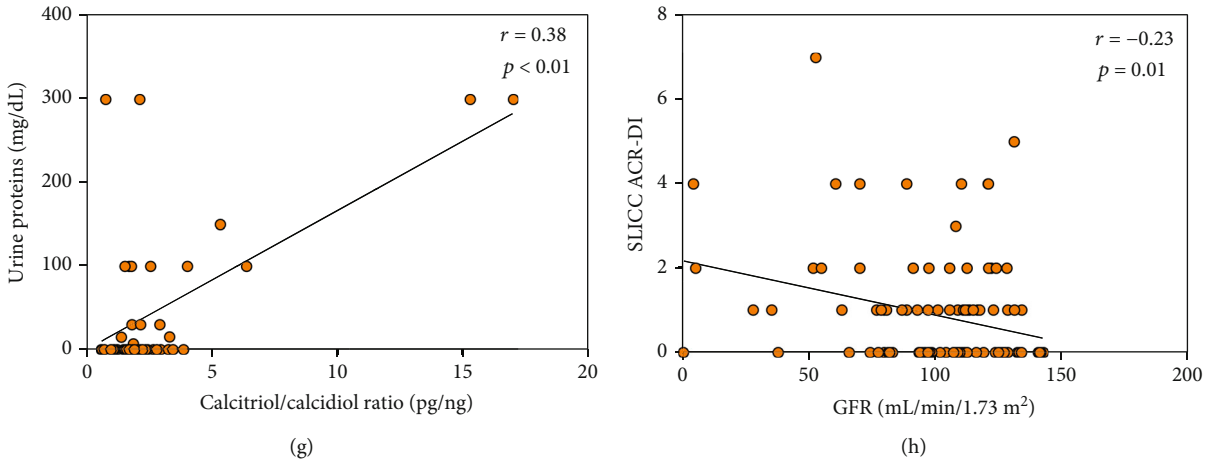


Figure 4: Correlations of vitamin D metabolite serum levels and vitamin D hydroxylation efficiency ratio with clinical and renal variables in SLE patients. (a) Correlation of calcidiol serum levels and calcitriol serum levels; (b) correlation of calcidiol serum levels and urine proteins; (c) correlation of calcidiol serum levels and serum calcium; (d) correlation of serum calcium and Mex-SLEDAI; (e) correlation of calcitriol serum levels with blood lymphocyte count; (f) correlation of calcitriol serum levels and GFR; (g) correlation of calcitriol/calcidiol ratio and urine proteins; (h) correlation of GFR with the SLICC ACR-DI score. r: Spearman's correlation coefficient; GFR: glomerular filtration rate.

activity = 38.5 pg/mL *vs.* renal inactivity = 39.6 pg/mL; $p = 0.42$) (Figure 3(e)).

Concerning vitamin D hydroxylation efficiency, the SLE patients with renal disease activity and calcidiol deficiency showed a higher calcidiol to calcitriol conversion rate (renal disease activity = 3.73 pg/ng [1.78–17.1] *vs.* renal disease inactivity = 2.73 pg/ng [1.71–11.1]; $p = 0.03$), followed by a significantly similar pattern in a calcidiol insufficiency status (renal disease activity = 2.0 pg/ng [1.69–2.17] *vs.* renal disease inactivity = 1.51 pg/ng [0.72–2.38]; $p = 0.01$), whereas in renal active SLE patients and renal inactive patients, both with calcidiol sufficiency, no significant differences were observed (renal disease activity = 1.14 pg/ng *vs.* renal disease inactivity = 0.98; $p = 0.93$) (Figure 3(f)). This similar pattern observed in the comparison of SLE patients with renal activity shows that the calcidiol deficiency and insufficiency are related to higher calcitriol serum levels and higher conversion rate of calcidiol to calcitriol compared to renal inactive SLE patients.

3.5. Association of Vitamin D Metabolites and Efficiency of Vitamin D Hydroxylation with SLE Clinical Variables. About the vitamin D metabolites, we observed that calcidiol serum levels were negatively correlated with the calcitriol serum levels ($r = -0.26$; $p = 0.001$; Figure 4(a)), a finding that was not observed in HS. As well, the calcidiol serum levels were negatively correlated with urine proteins (mg/dL) ($r = -0.39$; $p < 0.01$; Figure 4(b)) and positively correlated with serum calcium (mg/dL) ($r = 0.44$; $p = 0.01$; Figure 4(c)). Notably, the serum calcium correlated negatively with the de Mex-SLEDAI index ($r = -0.45$; $p = 0.01$; Figure 4(d)).

Calcitriol serum levels were positively correlated with the blood lymphocyte count ($r = 0.30$; $p < 0.001$; Figure 4(e)) and negatively correlated with the GFR ($r = -0.28$; $p = 0.001$; Figure 4(f)). Moreover, the calcitriol/calcidiol ratio was positively correlated with urine proteins ($r = 0.38$; $p < 0.01$; Figure 4(g)) and the GFR was negatively correlated with the SLICC ACR-DI ($r = -0.23$; $p = 0.01$; Figure 4(h)).

Based on the findings observed, we estimated the association of vitamin D metabolites and the calcitriol/calcidiol ratio with the risk to SLE and clinical and renal disease activities, using multiple logistic and linear regression models.

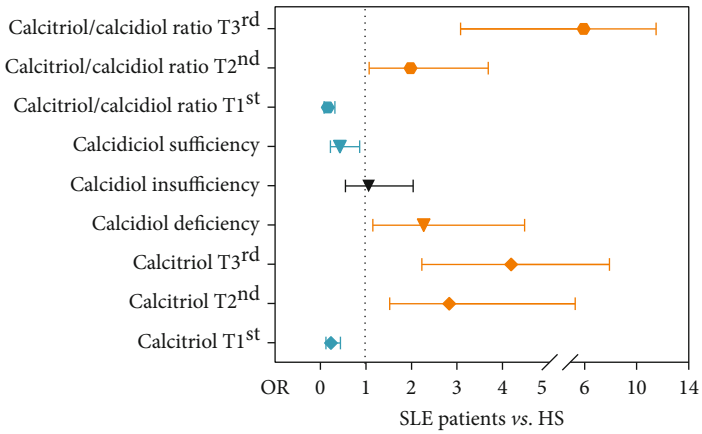
Regarding calcidiol serum levels, we observed that SLE patients with calcidiol deficiency had a 2.27-fold higher risk for SLE (OR = 2.27; $p < 0.01$), while SLE patients with calcidiol serum levels within sufficiency values have a 2.32-fold lower risk for SLE (OR = 0.43; $p < 0.01$) (Figure 5(a)).

About calcitriol serum levels, the SLE patients with calcitriol serum levels within the first tertile had a 4.34-fold lower risk for SLE ($T1^{st}$, OR = 0.23; $p < 0.001$), while the SLE patients with calcitriol levels within the second tertile had 2.83-fold higher risk for the disease ($T2^{nd}$, OR = 2.83; $p < 0.001$) and those within the third tertile had 4.19-fold higher risk to SLE ($T3^{rd}$, OR = 4.19; $p < 0.001$) (Figure 5(a)).

Concerning the efficiency of vitamin D hydroxylation assessed by the calcitriol/calcidiol ratio, SLE patients with a low-efficiency rate of vitamin D hydroxylation (first tertile) had 6.25-fold lower risk for SLE ($T1^{st}$; OR = 0.16; $p < 0.001$), while the SLE patients with an average efficiency rate of vitamin D hydroxylation (second tertile), who had 1.98-fold higher risk for SLE ($T2^{nd}$, OR = 1.98; $p = 0.01$), and those with a high calcitriol/calcidiol ratio (third tertile) had 5.93-fold higher risk for SLE ($T3^{rd}$, OR = 5.93; $p < 0.001$) (Figure 5(a)).

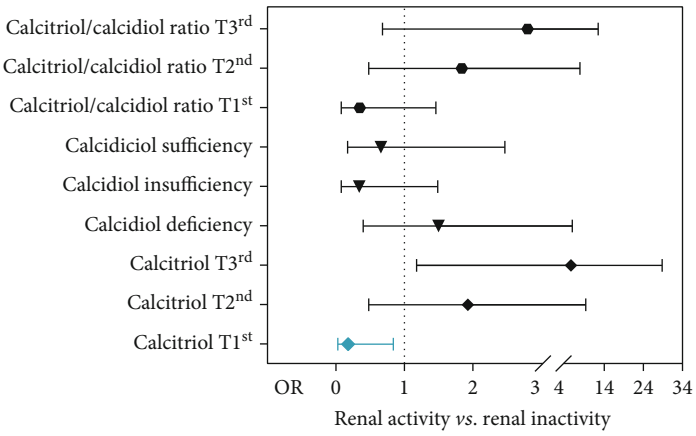
Besides, the SLE presence also was associated to low calcidiol serum levels (β coefficient = −3.6; $R^2 = 0.02$; $p = 0.01$), with high calcitriol serum levels (β coefficient = 14.4; $R^2 = 0.08$; $p < 0.001$), and a high calcidiol to calcitriol conversion rate (β coefficient = 1.93; $R^2 = 0.07$; $p < 0.001$) (data not shown).

Notably, SLE patients with low calcitriol serum values within the first tertile had 5.55-fold lower risk to renal



OR	95% CI	p
5.93	3.08–11.5	<0.001
1.98	1.07–3.69	0.01
0.16	0.08–0.32	<0.001
0.43	0.22–0.86	<0.01
1.06	0.55–2.04	0.84
2.27	1.15–4.49	<0.01
4.19	2.23–7.90	<0.001
2.83	1.52–5.27	<0.001
0.23	0.12–0.44	<0.001

(a)



OR	95% CI	p
2.80	0.68–12.5	0.10
1.84	0.48–7.84	0.31
0.35	0.08–1.46	0.10
0.66	0.17–2.47	0.49
0.34	0.08–1.49	0.09
1.50	0.40–5.84	0.49
5.50	1.18–28.8	0.01
1.93	0.48–9.31	0.30
0.18	0.03–0.04	0.01

(b)

Figure 5: Association of vitamin D metabolite serum levels and vitamin D hydroxylation efficiency ratio with the SLE and renal disease activity. (a) Association of vitamin D metabolite serum levels and calcitriol/calcidiol ratio with the SLE (SLE patients *vs.* HS); (b) association of vitamin D metabolite serum levels and calcitriol/calcidiol ratio with the presence of renal activity. HS: healthy subjects. OR: odds ratio, Fisher's exact test, blue (protective OR < 1) and orange (risk OR ≥ 1) graphics, p values < 0.05, 95% confidence interval (CI). Black graphics: not significant differences ($p > 0.05$) or high confidence intervals. Calcitriol/calcidiol ratio tertiles: $T3^{rd}$ (≥2.23 to 23.6 pg/ng), $T2^{nd}$ (≥1.36 to ≤2.23 pg/ng), and $T1^{st}$ (0.01 to <1.36 pg/ng). Calcidiol reference values: deficiency (<20 ng/mL), insufficiency (≥20 to <30 ng/mL), and sufficiency (≥30 ng/mL). Calcitriol tertiles: $T3^{rd}$ (≥48.7 to 157.3 pg/mL), $T2^{nd}$ (≥33.6 to <48.7 pg/mL), and $T1^{st}$ (0.33 to <33.6 pg/mL).

disease activity (OR = 0.18; $p = 0.01$) (Figure 5(b)). Finally, we observed that the presence of clinical and renal disease activities in SLE patients was associated with a significant high calcidiol to calcitriol conversion rate (β coefficient = 1.67, $R^2 = 0.04$, $p = 0.01$ and β coefficient = 1.76, $R^2 = 0.09$, $p < 0.01$, respectively) (data not shown).

4. Discussion

SLE patients have been reported to have a higher susceptibility to present vitamin D deficiency than the general population. We observed a higher frequency of calcidiol deficiency in SLE patients *vs.* HS, similar to other cross-sectional studies that reported lower calcidiol serum levels in SLE patients compared to HS [15, 31]. However, the prevalence of calcidiol deficiency varies from 3% to 67% in studies conducted in different SLE populations; these wide ranges in the differences in the calcidiol deficiency frequency observed between populations could be influenced by environmental and genetic factors that could modify the calcidiol serum levels: such as geographic latitude; low UV light exposure time; pharmacotherapy used such as glucocorticoids, which have a negative effect on vitamin D serum levels; additional comorbidities presented such as obesity, hypothyroidism, and renal damage; genetic polymorphisms such as those described in the *VDR* gene; older age of the participants included; and heterogeneous clinical disease activity status that presented the SLE patients in each study [7, 15, 18, 31].

Calcidiol deficiency in SLE patients has been associated with clinical disease activity and damage, with controversial findings [31–33]. In our study, no association between calcidiol levels with the clinical disease activity was observed; however, there was a trend of lower calcidiol serum values in active SLE patients and calcidiol serum levels were negatively correlated with calcitriol; this correlation pattern was not observed in HS. Notably, calcitriol serum levels were higher in the SLE patients compared to HS and high calcitriol serum levels in SLE patients were associated with the risk to the disease. Recently, very few studies in the literature have evaluated the calcitriol serum levels in SLE, despite being the biologically active metabolite of vitamin D [34]. However, it has been described that in a serum calcidiol-deficient status, calcitriol levels are often elevated due to local calcitriol synthesis in several organs and tissues to regulate the genes related to cellular proliferation and other homeostatic healthy important functions [24]. High calcitriol levels in pathological conditions could be indicative of active inflammatory disease; in a study of patients with granulomatous diseases, high calcitriol production by inflammatory cells has been associated with the inflammatory process, and in another study in patients with early diagnoses of tuberculous pleuritis, elevated calcitriol levels have been associated with active pulmonary tuberculosis [35].

Although similar data have not been reported in SLE or patients with other autoimmune diseases, high calcitriol serum levels have been described as a risk factor in other diseases such as cancer and allergies [35–38]. The antitumor properties of calcitriol have been reported in several studies; however, the calcitriol excess may not always be beneficial, particularly in cancer [36]. Murine studies about cancer showed that excess of calcitriol favors the stimulation of the metastasis of 4T1 mammary gland carcinoma in BALB/c mice [37]; besides, another study about the effects of calcitriol on immunity in 4T1 tumor-bearing mice showed an enhancement of Th2 response and stimulation of Th17 cell differentiation, with an increased local calcitriol synthesis in M2 tumor-associated macrophages. Thus, in cancer, the calcitriol may intensify the immunosuppression of the tumor niche, contributing to the stimulation of cancer progression [36].

In patients with asthma and chronic obstructive pulmonary disease (COPD), it has been reported that inflammatory processes dysregulate the vitamin D metabolism; these patients before and after a cholecalciferol supplementation showed lower calcidiol serum levels and higher calcitriol/calcidiol ratio; moreover, the patients with asthma presented higher calcitriol levels in comparison to controls. In these diseases, the expression of TNF-α, IL-1β, and TGF-β was increased and these proinflammatory cytokines have been reported to induce expression of the enzymes CYP24A1 and CYP27B1, which are related to calcidiol and calcitriol enzymatic hydroxylation, respectively [38].

Concerning the vitamin D hydroxylation efficiency evaluated in our study by the calcitriol/calcidiol ratio, the SLE patients showed a higher ratio than the HS, representing that SLE patients are converting more calcidiol to calcitriol, like the pattern described in patients with asthma and COPD [38]. In our study, this pattern of high vitamin D hydroxylation efficiency ratio and high calcitriol serum levels was more evidently observed in calcidiol-deficient SLE patients with clinical and renal disease activities. According to our findings, Pasquali et al. reported in subjects with risk to renal disease and calcidiol deficiency a similar pattern of high calcitriol/calcidiol ratio and high calcitriol serum levels; besides conforming that the calcidiol sufficiency was reached, the calcitriol/calcidiol ratio was lower compared to those of calcidiol-deficient subjects [20], highlighting a possible compensatory regulation mechanism that promotes a higher calcitriol synthesis when the calcidiol deficiency is presented.

Regarding this, it has been described that the inflammatory state modulated by TNF-α and IFN induces via Toll-like receptors (TLRs) the upregulation of the VDR expression on the macrophage surface, inducing the activity of the CYP27B1 enzyme related to the conversion of calcidiol to calcitriol in situ, which has a costimulatory effect on lymphocyte polarization [5, 24, 39, 40]. This event might support that the immune cells follow a biphasic dose response of several cytokines and nutrients, as suggested for calcitriol concentrations [41]. In a murine model of experimental autoimmune encephalomyelitis (EAE), a moderate diet in vitamin D (1500 IU/kg food) decreased the severity of EAE, while high doses of cholecalciferol supplementation (75000 IU/kg food) enhance activation, proinflammatory differentiation of T cells, and phagocytic activity of peripheral myeloid antigen-presenting cells, characterized by an enhanced surface expression of MHC class II and costimulatory molecules CD40, CD80, and CD86 [42]. Additionally, mice with high vitamin D levels showed in the central nervous system higher IFN-γ and IL-17 levels with lower frequency of Treg cells [42, 43]. Hence, the beneficial effect of vitamin D may be achieved at moderate doses.

Some studies have reported that calcitriol administration may prevent strong Th1 responses, whereas its effects on Th2 cells are more complex and not fully elucidated [11, 44, 45]. Also, as previously mentioned, a possible biphasic effect of vitamin D on allergies has been described; vitamin D deficiency may be related to allergic reactions; however, it has been hypothesized that the vitamin D excess leads to an increased allergy risk with a Th2 response predominance and high production of specific IgE against the allergen by the B cells, resulting in an acute inflammatory response [44, 45], maybe modulated by the pattern of high calcitriol when the calcidiol deficiency is presented. Notably, in SLE, a Th2 response and activation of B cells have classically been associated with the pathophysiology of the disease.

The high calcitriol serum levels observed in our study could be promoting an increase in the Th2 cell response in the SLE patients. A subtle balance between Th1 and Th2 cells is provided under normal conditions. Nevertheless, in autoimmunity, a strong Th2 predominance leads to pathologic conditions [45]. Several studies suggest that SLE is a Th2 profile-driven disease [4, 6] characterized by IL-4, IL-5, IL-6, IL-10, and IL-13 cytokines. In murine models, IL-4 promoted the survival of autoreactive B cells and the high expression of IL-5 promotes the proliferation and differential signaling of self-antigen-activated B cell [5]. Regarding

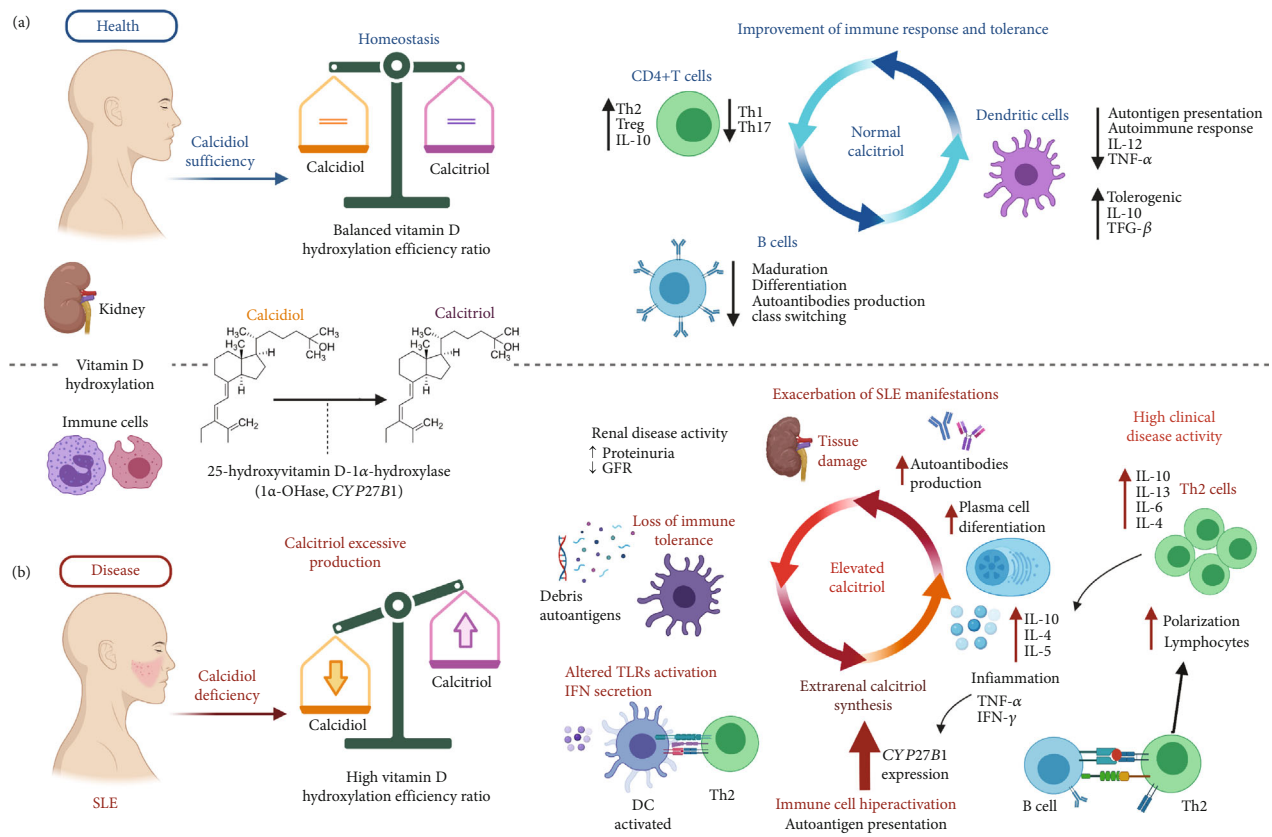


Figure 6: Biphasic effect of calcitriol on health and autoimmunity. (a) In health, the beneficial effect of calcitriol could be achieved at moderate concentrations presented in the calcidiol sufficiency status. Adequate concentrations of calcidiol and calcitriol (vitamin D sufficiency) allow adequate immunomodulation that improves the immune response and tolerance, favoring polarization towards the tolerogenic Th profiles, reducing the maturation of autoreactive B cells and the antigenic presentation of the dendritic cells, with an increase of IL-10 and TGF-β cytokines. (b) In SLE, lower calcidiol serum levels (vitamin D deficiency) increase the vitamin D hydroxylation efficiency ratio and the extrarenal calcitriol synthesis; this event may occur due to a compensatory mechanism where the immune cell system in a proinflammatory autoimmune context increases the extrarenal hyperactivity of the CYP27B1 enzyme, contributing to an increase in the calcitriol serum concentrations by calcidiol hydroxylation and promotes the exacerbation of the pathological autoimmune response, characterized by a positive feedback loop of the aberrant self-antigenic presentation, the plasma cell differentiation, autoantibody production, autoreactive Th2 polarization, tolerance loss, and damage to tissues.

IL-6 and IL-10, these cytokines have been associated with high clinical disease activity in SLE, while IL-13 induces the expression of IgE, IL-6, and surface antigen such as CD23, CD71, and MHC II, which together could contribute to the exacerbation of the disease [5].

This pattern of low calcidiol and high calcitriol serum levels observed in our study in SLE patients could be influenced by a high polyclonal B cell activation in active SLE patients. The activation of human B cells induces the CYP27B1 enzyme expression; these cells secrete large amounts of calcitriol, enhancing IL-10 expression from activated B cells more than threefold [11, 46]. According to the biphasic functions of calcitriol, IL-10 is another molecule that has a biphasic function, which could be either protective or pathologic following a concentration gradient; in particular, high IL-10 serum levels in SLE are considered pathogenic. Increased IL-10 production induced by calcitriol may promote the B cell differentiation into plasmablasts, promoting B cells' hyperactivity, autoantibody production, and the antibody class switch to IgA, IgG, and IgM [5, 13, 46, 47].

Notably, in our study, the SLE patients with renal disease activity also showed the same pattern of lower calcidiol serum levels with higher vitamin D hydroxylation ratio and higher calcitriol serum levels in comparison to SLE patients without renal activity. According to this, in SLE patients from Poland, 37% of patients with renal disorder showed lower calcidiol serum levels than patients without renal disorder ($p = 0.006$) [48]. In another study conducted in African-American and Caucasian SLE patients, 18% of patients showed low calcidiol serum levels (<10 ng/mL) and the presence of renal disease was a predictor to lower calcidiol serum levels (OR = 13.3, 95% CI 2.3-76.7, $p < 0.001$) [49], while in pediatric SLE patients from the U.S., the presence of proteinuria (urine protein/creatinine ratio $\geq$ 0.5) was associated with a decrease of 13 ng/mL of calcidiol and 19.7-fold increased risk of deficient calcidiol serum levels $\leq$ 10 ng/mL (95% CI 1.8-944.5) [50]. Nevertheless, in these previous studies, calcitriol serum levels were not quantified to be able to contrast the pattern observed in our results.

In our study, high calcitriol/calcidiol ratio scores and high calcitriol serum levels were negatively correlated with

the GFR in SLE patients. These observed findings highlight that the kidney is not the only source of calcitriol in patients with renal compromise, is possible that the calcitriol/calcidiol ratio could represent the extrarenal synthesis of calcitriol by immune cells [20]; this event could explain why the SLE patients with renal disease activity had higher calcitriol serum levels in comparison to inactive renal patients.

Regarding this, immune cells such as monocytes, DCs, and B cells can produce calcitriol by the local CYP27B1 expression [45]. Calcitriol enhances the polarization to the Th2 phenotype [11], and the contributions of Th2 cytokines to the renal disease include the production of IL-6 and IL-4 by activated basophils that leads to autoantibody deposition in the kidney via enhanced Th2 response and B cell activation, promoting the release of Th2 cytokines such as IL-10, IL-13, and IL-6; the influx of inflammatory cells; and renal manifestation in active SLE or SLE patients with LN, compared to patients without renal disorders [4, 5].

Therefore, although calcidiol is considered the best indicator of the serum vitamin D status, its deficiency could not reflect calcitriol's serum status [20]. Despite that the half-life of calcitriol in the circulation is very short (12–36 h), its concentration appears to be relatively steady because, after a negative or positive stimulus, several weeks are necessary to return to the prestimulus calcitriol serum range. This indicates that one blood measurement at a specific time point is sufficient to estimate the range in which circulating calcitriol concentrations will be seen over several weeks [44]. Hence, the implementation of the quantification of both vitamin D metabolites, calcidiol and calcitriol, together with the evaluation of the calcitriol/calcidiol ratio, could be important biomarkers in SLE to elucidate in a better way the complex biological regulation of the vitamin D serum status in the clinical disease and renal activities in SLE.

Based on our results and previous findings reported about calcitriol, we hypothesized that the pattern of high calcitriol serum levels and increased synthesis of calcitriol in calcidiol-deficient SLE patients could be due to a compensatory mechanism to regulate the low amount of available calcidiol serum, and in an autoimmune context, the increase of calcitriol serum concentration in calcidiol-deficient SLE patients that present clinical and renal activity could contribute to the exacerbation of the autoimmune response. Notably, in physiological conditions, calcitriol's beneficial effects could be achieved at moderate calcitriol concentrations presented in the calcidiol sufficiency status (Figure 6).

According to our results, the strength of the present study was that the sample of SLE patients and HS evaluated was homogeneous in the following characteristics: all participants were female, from the same geographic area (western Mexico), classified as the Mexican-mestizo population with three ancestors in the same geographic region, and of similar age, which reduces the bias regarding environmental and genetic factors of ancestry that could influence the results. Nevertheless, our methodological constraints were that our cross-sectional study design limited us by simply showing an association between high calcitriol serum levels and the calcitriol/calcidiol ratio with SLE and renal disease activities. Therefore, we do not suggest causality because our study only provides information on a specific time point. Moreover, the other limitations of the present study were that we did not quantify the serum levels of proinflammatory cytokine associated with pathogenic Th2 response in SLE and the molecules related to vitamin D metabolism, such as cholecalciferol, PTH, and phosphorus, that could also influence the vitamin D serum levels. Furthermore, to test our hypothesis, we were not able to evaluate the expression at the mRNA, protein level, and enzymatic activity of the vitamin D hydroxylase enzymes involved in the catabolism of calcidiol such as CYP24A1 and the calcitriol renal and extrarenal synthesis such as CYP27B1 in the kidney and leucocytes from SLE patients and HS with vitamin D deficiency.

Therefore, further studies in SLE focused on these points will be necessary to perform, to assess the causality of the relationship of low calcidiol, high-calcidiol-to-calcitriol conversion rate, and high calcitriol serum levels in SLE patients with clinical and renal disease activities described in the present cross-sectional study. These future studies will help to support the clinical and translational interventions with vitamin D supplementation in subsequent studies conducted in patients with autoimmune diseases.

5. Conclusions

In conclusion, a pattern of calcidiol deficiency with high calcitriol serum levels and high vitamin D hydroxylation efficiency ratio was associated with disease risk in SLE patients.

Abbreviations

ACR:	American College of Rheumatology
GFR:	Glomerular filtration rate
HS:	Healthy subjects
Mex-SLEDAI:	Mexican Systemic Lupus Erythematosus Disease Activity Index
mL:	Milliliter
ng:	Nanograms
pg:	Picograms
SLE:	Systemic lupus erythematosus
SLICC-DI:	Systemic Lupus International Collaborating Clinics Damage Index
T:	Tertile.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

The funding sources were not involved in any step of the study.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All

authors approved the final version of the manuscript. Figure 6 was created with http://BioRender.com/.

Authors' Contributions

Conceptualization was done by U.D.C.M.; the methodology was done by I.P.R., A.I.R.B., and B.V.L.; formal analysis was performed by M.R.M.M., E.M.L., Y.F.M.S., I.P.R., S.C.C., and M.M.B.; the investigation was done by M.R.M.M. and A.I.R.B.; resources were gathered by U.D.C.M.; writing—original draft preparation was performed by M.R.M.M.; writing—review and editing were done by U.D.C.M., E.O.R., J.F.M.V., A.I.R.B., B.V.L., E.M.L., Y.F.M.S., I.P.R., S.C.C., and M.M.B.; visualization was done by U.D.C.M; supervision was done by U.D.C.M; project administration was done by U.D.C.M; funding acquisition was done by U.D.C.M. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This study was supported in part by Grant no. UDG-PTC 1401 for Ulises de la Cruz-Mosso, PhD (U.D.C.M.), from the *Apoyo a la Incorporación de NPTC SEP-PRODEP 2018* and in part by Grant *PRO-SNI 2017-2020* (U.D.C.M.) from *Programa de Apoyo a la Mejora en las Condiciones de Producción de los Miembros del SNI y SNCA* 2017–2019 and the *"Programa de Impulso a la Investigación (PIN) 2020"* (U.D.C.M.), from the University of Guadalajara.

References

[1] K. S. Yap, M. Northcott, A. B. Y. Hoi, E. F. Morand, and M. Nikpour, "Association of low vitamin D with high disease activity in an Australian systemic lupus erythematosus cohort," *Lupus Science & Medicine*, vol. 2, no. 1, article e000064, p. 6, 2015.

[2] Z. Liu and A. Davidson, "Taming lupus–a new understanding of pathogenesis is leading to clinical advances," *Nature Medicine*, vol. 18, no. 6, pp. 871–882, 2012.

[3] G. C. Tsokos, M. S. Lo, P. C. Reis, and K. E. Sullivan, "New insights into the immunopathogenesis of systemic lupus erythematosus," *Nature Reviews Rheumatology*, vol. 12, no. 12, pp. 716–730, 2016.

[4] M. Selvaraja, M. Abdullah, M. Arip, V. K. Chin, A. Shah, and S. Amin Nordin, "Elevated interleukin-25 and its association to Th2 cytokines in systemic lupus erythematosus with lupus nephritis," *PLoS One*, vol. 14, no. 11, p. 17, 2019.

[5] F. Muhammad Yusoff, K. K. Wong, and N. Mohd Redzwan, "Th1, Th2, and Th17 cytokines in systemic lupus erythematosus," *Autoimmunity*, vol. 53, no. 1, pp. 8–20, 2020.

[6] K. Miyake, M. Akahoshi, and H. Nakashima, "Th subset balance in lupus nephritis," *Journal of Biomedicine and Biotechnoly*, vol. 2011, article 980286, 7 pages, 2011.

[7] R. Miskovic, A. Plavsic, S. Raskovic, Z. Jovicic, and J. Bolpacic, "Vitamin D status in patients with systemic lupus erythematosus in Serbia: correlation with disease activity and clinical manifestations," *Macedonian Journal of Medical Sciences*, vol. 3, no. 2, pp. 256–261, 2015.

[8] A. Mak, "The impact of vitamin D on the immunopathophysiology, disease activity, and extra-musculoskeletal manifestations of systemic lupus erythematosus," *International Journal of Molecular Sciences*, vol. 19, no. 8, p. 2355, 2018.

[9] M. R. Meza-Meza, A. I. Ruiz-Ballesteros, and U. De la Cruz-Mosso, "Functional effects of vitamin D: from nutrient to immunomodulator," *Critical Reviews in Food Science and Nutrition*, pp. 1–21, 2020.

[10] Z. H. Zheng, C. C. Gao, Z. Z. Wu et al., "High prevalence of hypovitaminosis D of patients with autoimmune rheumatic diseases in China," *American Journal of Clinical and Experimental Immunology*, vol. 5, no. 3, pp. 48–54, 2016.

[11] W. Dankers, E. M. Colin, J. P. van Hamburg, and E. Lubberts, "Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential," *Frontiers in Immunology*, vol. 7, p. 697, 2017.

[12] S. W. Kang, S. H. Kim, N. Lee et al., "1,25-Dihyroxyvitamin D3PromotesFOXP3Expression via binding to vitamin D response elements in its conserved noncoding sequence region," *Journal of Immunology*, vol. 188, no. 11, pp. 5276–5282, 2012.

[13] J. Godsell, I. Rudloff, R. Kandane-Rathnayake et al., "Clinical associations of IL-10 and IL-37 in systemic lupus erythematosus," *Scientific Reports*, vol. 6, no. 1, article 34604, p. 10, 2016.

[14] M. Eloi, D. V. Horvath, J. C. Ortega et al., "25-Hydroxivitamin D serum concentration, not free and bioavailable vitamin D, is associated with disease activity in systemic lupus erythematosus patients," *PLoS One*, vol. 12, no. 1, article e0170323, p. 10, 2017.

[15] C. C. Gao, S. Y. Liu, Z. Z. Wu et al., "Severe vitamin D deficiency increases the risk for moderate to severe disease activity in Chinese patients with SLE," *Lupus*, vol. 25, no. 11, pp. 1224–1229, 2016.

[16] S. G. Ong and H. J. Ding, "Vitamin D status in a monocentric cohort of systemic lupus erythematosus (SLE) patients and correlations with clinical and immunological profile," *Medical Journal of Malaysia*, vol. 74, no. 6, pp. 492–498, 2019.

[17] S. Azrielant and Y. Shoenfeld, "Eppur Si Muove: vitamin D is essential in preventing and modulating SLE," *Lupus*, vol. 25, no. 6, pp. 563–572, 2016.

[18] M. García-Carrasco, C. Mendoza-Pinto, I. Etchegaray-Morales et al., "Insuficiencia y deficiencia de vitamina D en pacientes mexicanas con lupus eritematoso sistemico: prevalencia y relacion con actividad de la enfermedad," *Reumatología Clínica*, vol. 13, no. 2, pp. 97–101, 2017.

[19] Y. Shoenfeld, R. Giacomelli, S. Azrielant, O. Berardicurti, J. A. Reynolds, and I. N. Bruce, "Vitamin D and systemic lupus erythematosus - the hype and the hope," *Autoimmunity Reviews*, vol. 17, no. 1, pp. 19–23, 2018.

[20] M. Pasquali, L. Tartaglione, S. Rotondi et al., "Calcitriol/calcifediol ratio: an indicator of vitamin D hydroxylation efficiency?," *BBA Clinical*, vol. 3, pp. 251–256, 2015.

[21] M. C. Hochberg, "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus," *Arthritis & Rheumatism*, vol. 40, no. 9, pp. 1725–1734, 1997.

[22] A. G. Uribe, L. M. Vilá, G. McGwin, M. L. Sanchez, J. D. Reveille, and G. S. Alarcón, "The systemic lupus activity measure-revised, the Mexican Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and a modified SLEDAI-2K are adequate instruments to measure disease activity in systemic

lupus erythematosus," *Journal of Rheumatology*, vol. 31, no. 10, pp. 1934–1940, 2004.

[23] D. Gladman, E. Ginzler, C. Goldsmith et al., "The development and initial validation of the systemic lupus international collaborating clinics/American college of rheumatology damage index for systemic lupus erythematosus," *Arthritis & Rheumatism*, vol. 39, no. 3, pp. 363–369, 1996.

[24] M. F. Holick, "Vitamin D deficiency," *New England Journal of Medicine*, vol. 357, no. 3, pp. 266–281, 2007.

[25] A. Celikbilek, A. Y. Gocmen, G. Zararsiz et al., "Serum levels of vitamin D, vitamin D-binding protein and vitamin D receptor in migraine patients from central Anatolia region," *International Journal of Clinical Practice*, vol. 68, no. 10, pp. 1272–1277, 2014.

[26] L. Ovesen, C. Brot, and J. Jakobsen, "Food contents and biological activity of 25-hydroxyvitamin D: a vitamin D metabolite to be reckoned with?," *Annals of Nutrition and Metabolism*, vol. 47, no. 3-4, pp. 107–113, 2003.

[27] E. Ávila, D. Barrera, and L. Díaz, "Acciones calciotrópicas de la hormona paratiroidea y del sistema endocrino de la vitamina D," *Revista de Investigación Clínica*, vol. 59, no. 4, pp. 306–317, 2007.

[28] A. S. Levey, L. A. Stevens, C. H. Schmid et al., "A new equation to estimate glomerular filtration rate," *Annals of Internal Medicine*, vol. 150, no. 9, pp. 604–612, 2009.

[29] I. H. de Boer, M. L. Caramori, J. C. Chan et al., "KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease," *Kidney International*, vol. 98, no. 4, pp. S1–S115, 2020.

[30] M. García-Carrasco, C. Mendoza-Pinto, J. Ayón-Aguilar et al., "Serum levels of vitamin D in systemic lupus erythematosus patients (SLE) and their relationship with disease activity: longitudinal study," *Gaceta Médica de México*, vol. 152, suppl. 2, pp. 32–37, 2016.

[31] N. M. Abaza, R. M. el-Mallah, A. Shaaban et al., "Vitamin D deficiency in Egyptian systemic lupus erythematosus patients: how prevalent and does it impact disease activity?," *Integrative Medicine Insights*, vol. 11, article IMI.S40035, pp. 27–33, 2016.

[32] V. Z. C. Borba, J. G. H. Vieira, T. Kasamatsu, S. C. Radominski, E. I. Sato, and M. Lazaretti-Castro, "Vitamin D deficiency in patients with active systemic lupus erythematosus," *Osteoporosis International*, vol. 20, no. 3, pp. 427–433, 2009.

[33] S. S. Yeap, A. Z. Othman, A. A. Zain, and S. P. Chan, "Vitamin D levels: its relationship to bone mineral density response and disease activity in premenopausal Malaysian systemic lupus erythematosus patients on corticosteroids," *International Journal of Rheumatic Diseases*, vol. 15, no. 1, pp. 17–24, 2012.

[34] K. Müller, N. J. Kriegbaum, B. Baslund, O. H. Sørensen, M. Thymann, and K. Bentzen, "Vitamin D3 metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin D3 in patients with systemic lupus erythematosus," *Clinical Rheumatology*, vol. 14, no. 4, pp. 397–400, 1995.

[35] P. F. Barnes, R. L. Modlin, D. D. Bikle, and J. S. Adams, "Transpleural gradient of 1, 25-dihydroxyvitamin D in tuberculous pleuritis," *The Journal Clinical Investigation*, vol. 83, no. 5, pp. 1527–1532, 1989.

[36] A. Anisiewicz, N. Łabędź, I. Krauze, and J. Wietrzyk, "Calcitriol in the presence of conditioned media from metastatic breast cancer cells enhances ex vivo polarization of M2 alternative murine bone marrow-derived macrophages," *Cancers*, vol. 12, no. 11, article 3485, p. 26, 2020.

[37] A. Anisiewicz, A. Pawlik, B. Filip-Psurska et al., "Unfavorable effect of calcitriol and its low-calcemic analogs on metastasis of 4T1 mouse mammary gland cancer," *International Journal of Oncology*, vol. 52, no. 1, pp. 103–126, 2017.

[38] D. A. Jolliffe, C. Stefanidis, Z. Wang et al., "Vitamin D metabolism is dysregulated in asthma and chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 202, no. 3, pp. 371–382, 2020.

[39] K. Müller, P. Oxholm, O. H. Sørensen, M. Thymann, M. Høier-Madsen, and K. Bendtzen, "Abnormal vitamin D3 metabolism in patients with primary Sjogren's syndrome," *Annals of the Rheumatic Diseases*, vol. 49, no. 9, pp. 682–684, 1990.

[40] D. Bikle, "Nonclassic actions of vitamin D," *The Journal of Clinical Endocrinology & Metabolism*, vol. 94, no. 1, pp. 26–34, 2009.

[41] G. Csaba, "Hormesis and immunity: a review," *Acta Microbiologica et Immunologica Hungarica*, vol. 66, no. 2, pp. 155–168, 2019.

[42] D. Häusler, S. Torke, E. Peelen et al., "High dose vitamin D exacerbates central nervous system autoimmunity by raising T-cell excitatory calcium," *Brain*, vol. 142, no. 9, pp. 2737–2755, 2019.

[43] V. Sedeño Monge, E. A. Fabre Palacios, C. López García, and M. . L. Meza Jiménez, "Nutrientes y alimentos en la esclerosis múltiple," *Archivos Latinoamericanos de Nutrición*, vol. 70, no. 1, pp. 60–74, 2020.

[44] A. Zittermann, S. S. Schleithoff, S. Frisch et al., "Circulating calcitriol concentrations and total mortality," *Clinical Chemistry*, vol. 55, no. 6, pp. 1163–1170, 2009.

[45] A. Zittermann, G. Tenderich, and R. Koerfer, "Vitamin D and the adaptive immune system with special emphasis to allergic reactions and allograft rejection," *Inflammation & Allergy Drug Targets*, vol. 8, no. 2, pp. 161–168, 2009.

[46] G. Heine, U. Niesner, H. D. Chang et al., "1,25-Dihydroxyvitamin D3promotes IL-10 production in human B cells," *European Journal of Immunology*, vol. 38, no. 8, pp. 2210–2218, 2008.

[47] H. Peng, W. Wang, M. Zhou, R. Li, H. F. Pan, and D. Q. Ye, "Role of interleukin-10 and interleukin-10 receptor in systemic lupus erythematosus," *Clinical Rheumatology*, vol. 32, no. 9, pp. 1255–1266, 2013.

[48] J. Bogaczewicz, A. Sysa-Jedrzejowska, C. Arkuszewska et al., "Vitamin D status in systemic lupus erythematosus patients and its association with selected clinical and laboratory parameters," *Lupus*, vol. 21, no. 5, pp. 477–484, 2012.

[49] D. L. Kamen, G. S. Cooper, H. Bouali, S. R. Shaftman, B. W. Hollis, and G. S. Gilkeson, "Vitamin D deficiency in systemic lupus erythematosus," *Autoimmunity Reviews*, vol. 5, no. 2, pp. 114–117, 2006.

[50] A. B. Robinson, M. Thierry-Palmer, K. L. Gibson, and C. E. Rabinovich, "Disease activity, proteinuria, and vitamin D status in children with systemic lupus erythematosus and juvenile dermatomyositis," *The Journal of Pediatrics*, vol. 160, no. 2, pp. 297–302, 2012.

Hindawi
Journal of Immunology Research
Volume 2021, Article ID 7523997, 11 pages
https://doi.org/10.1155/2021/7523997

Research Article

Polymorphisms CYP2R1 rs10766197 and CYP27B1 rs10877012 in Multiple Sclerosis: A Case-Control Study

A. Martinez-Hernandez,[1] E. E. Perez-Guerrero,[2] M. A. Macias-Islas,[3] C. A. Nava-Valdivia,[4] A. Villagomez-Vega,[5] B. Contreras-Haro,[5] Y. E. Garcia-Ortega,[6] Y. Esparza-Guerrero,[1] S. G. Gallardo-Moya,[1] J. I. Gamez-Nava,[1,7] L. Gonzalez-Lopez,[1,7] E. Oliva-Flores,[1] N. A. Rodriguez-Jimenez,[8] F. Cortes-Enriquez,[9] and A. M. Saldaña-Cruz[8]

[1]*Programa de Doctorado en Farmacología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico*
[2]*Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico*
[3]*Departamento de Neurociencias, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico*
[4]*Departamento de Microbiología Y Patología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico*
[5]*Departamento de Ciencias Biomédicas, Centro Universitario de Tonalá, Universidad de Guadalajara, Tonalá, Mexico*
[6]*Hospital de Especialidades, Centro Médico Nacional de Occidente, Guadalajara, Jalisco, Mexico*
[7]*Doctorado en Salud Pública, Departamento de Salud Pública, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico*
[8]*Instituto de Terapéutica Experimental Y Clínica, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico*
[9]*Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico*

Correspondence should be addressed to A. M. Saldaña-Cruz; ana.saldanac@academicos.udg.mx

Received 29 October 2021; Accepted 17 November 2021; Published 23 December 2021

Academic Editor: Vlad Padureanu

Background. Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease. Low vitamin D levels have been reported to be a risk factor for MS, and genetic variances could be implicated. The aim of this study was to evaluate the association of MS with rs10766197 polymorphism of *CYP2R1* gene and rs10877012 polymorphism of *CYP27B1* gene. The second aim was to analyse whether these polymorphisms are associated with the severity of the progression of MS. *Material and Methods*. In a case-control study, we included 116 MS patients and 226 controls, all of whom were Mexican Mestizo. MS was diagnosed by McDonald criteria (2017). A complete neurological evaluation was performed to evaluate the severity of disease progression. Serum 25-hydroxyvitamin D [25(OH) vitamin D] levels were measured by ELISA. Single nucleotide polymorphisms rs10766197 of *CYP2R1* gene and rs10877012 SNP of *CYP27B1* gene were genotyped by real-time PCR. *Results*. Serum 25(OH) vitamin D levels were lower in MS patients than in controls ($p = 0.009$). No differences were observed between serum 25(OH) vitamin D levels of MS patients with severe progression compared to low progression ($p = 0.88$). A higher frequency of the A allele of *CYP2R1* rs10766197 was observed between MS patients and controls ($p = 0.05$). No differences were observed in the frequency of T allele of *CYP27B1* rs10877012 ($p = 0.65$). In subanalysis, patients with GA + AA genotypes of *CYP2R1* rs10766197 had an increased risk of MS compared to controls ($p = 0.03$). No increased risk was observed in GT + TT genotypes of *CYP27B1* rs10877012

($p = 0.63$). No differences were observed in allele frequencies of either polymorphism between patients with severe vs. low disease progression. *Conclusion*. Lower serum 25(OH) vitamin D levels were observed in MS patients than in controls, although these levels were not associated with disease progression. Carriers of GA + AA genotypes of *CYP2R1* rs10766197 had an increased risk of MS. None of these polymorphisms was associated with severe progression of MS.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) [1]. Typically, MS affects the brain, spinal cord, and optic nerves, with inflammatory lesions derived from probable autoimmune mechanisms; these lesions are followed by axonal and grey-matter neurodegenerative processes associated with progressive worsening of disability. Most patients experience their first symptoms between the ages of 20 and 40 years. MS is considered the leading cause of disability in young people, excluding injuries caused by traffic accidents [2]. In Mexico, the prevalence of MS is 12 to 15 per 100,000 inhabitants [3].

There are four categories of MS: clinically isolated syndrome (CIS), relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS), with RRMS accounting for 85% of patients [4]. The World Health Organization (WHO) estimates that more than two million people worldwide suffer from MS [5].

Clinical symptoms of MS may include motor dysfunction, tremor, dysmetria, cerebellar ataxia, nystagmus, diplopia, hypoesthesia, blindness (usually unilateral), and sphincter disturbances such as retention or incontinence; approximately 45 to 60% of patients will develop cognitive decline and neuropsychiatric symptoms [6]. Motor disorders are the most common symptoms in the course of the disease which lead to motor disability [7].

MS is currently considered a very heterogeneous disorder in which both genetic susceptibility and environmental exposures are strongly implicated in T cell activation and pathogenesis [8]. Epstein-Barr virus, cytomegalovirus, and mononucleosis infections; smoking; adolescent obesity; night work; low sun exposure; and 25-hydroxyvitamin D (25(OH) vitamin D) < 30 ng/mL are recognized as risk factors for MS [9]. Vitamin D has been proposed as a serum biomarker in several clinical conditions, including neurodegenerative disorders; additionally, several inflammatory and cardiovascular markers reflect the immunomodulatory function of vitamin D [10]. It has been hypothesized that lower 25(OH) vitamin D levels are associated with the risk of MS [9, 11]. Possible explanations for the association between MS risk and altered vitamin D status alterations include the role of vitamin D in CD4+ T cell balance and remyelination processes, B lymphocyte class switching, immunoglobulin production, and proinflammatory interleukin secretion [12, 13] as well as the presence of vitamin D-responsive elements (VDREs) in the promoter regions of various MS-associated genes [14].

The metabolism of vitamin D, or cholecalciferol, is carried out by enzymes of the cytochrome p450 family, which catalyse a series of hydroxylation reactions to produce different active compounds [15]. CYP2R1 is responsible for the primary hepatic metabolism of vitamin D to 25(OH) vitamin D, which is subsequently converted to 1,25-dihydroxyvitamin D, the primary bioactive vitamin D metabolite, by CYP27B1in the kidney [15–17]. By binding to the vitamin D receptor, a member of the nuclear receptor superfamily, this metabolite mediates functions in multiple classes of cells, including immune cells [18].

Therefore, it is of great importance to study genetic factors that may modify 25(OH) vitamin D levels and have consequential impacts on the disease. The *CYP2R1* gene, found on chromosome 11p15.2, encodes the enzyme 25-hydroxylase, which is involved in the first step of vitamin D hydroxylation activation. The rs10766197 polymorphism of the *CYP2R1* gene has been shown to be an important factor with a major impact on vitamin D levels [19, 20]. The *CYP27B1* gene is located on chromosome 12q13.1-q13.3 and encodes the enzyme 1-alpha-hydroxylase. The rs10877012 polymorphism has been associated with reduced vitamin D levels; thus, together with the rs10766197 polymorphism, it is a promising object of research due to its potential influence in patients with MS (Ramos 2008) [20].

Prior research has found an association the rs10766197 SNP of the *CYP2R1* gene and the risk of MS as well as the progression of the disease [19]; but there has been no prior research on the rs10877012 SNP of the *CYP27B1* gene in patients with MS.

Therefore, the first aim of this study was to evaluate the association of MS with the rs10766197 polymorphism of the *CYP2R1* gene and the rs10877012 polymorphism of the *CYP27B1* gene. The second aim was to analyse whether these polymorphisms are associated with the severity of MS progression.

2. Materials and Methods

2.1. Study Design. The design of this study is a Case-Control Study, in which the cases were patients with RRMS and the controls were healthy patients without MS, residents of the Guadalajara metropolitan area.

2.2. Clinical Setting. This study included 116 RRMS patients and 226 controls. The MS cases were patients treated at the Mexican Association for Multiple Sclerosis A.C. in Guadalajara, Mexico, from January 24, 2019, to August 31, 2021.

2.3. Study Subjects. All individuals were aged ≥ 18 years and were Mexican-Mestizo as defined by the Mexican National Institute of Anthropology and History: "individuals who were born in Mexico, of the original autochthonous inhabitants of the region and individuals who were mainly Spaniards" [21].

Controls were selected randomly from the population available at the Clinical and Experimental Therapeutic Institute, consisting of 185 women and 41 men. Controls were included if they had no history of inflammatory or autoimmune disorders, and only one person per family was recruited. Controls were included to compare serum 25(OH) vitamin D levels and the genotypes of the polymorphisms.

2.4. Clinical Assessments. Patients with RRMS were included and assessed by an experienced neurologist and based on a previous history of disease and physical examination. Complete neurological examination assured that the patients fulfilled the 2017 McDonald clinical criteria. We excluded patients with kidney disease, liver disease, other uncontrolled autoimmune or psychiatric diseases, and current pregnancy.

Functional systems assessment was used to establish the disability score according to Kurtzke's Extended Disability Status Scale (EDSS) [22]. To ensure a correct diagnosis, we performed 1.5-tesla MRI with conventional T1, gadolinium-enhanced T1, T2, and fluid-attenuated inversion recovery (FLAIR) sequences and confirmed the presence of typical round hyperintense lesions in T2 and FLAIR sequences and hypointensity in T1 with or without enhancement distributed in a different location; the time of evolution differed for each patient [23]. Oligoclonal bands were not considered mandatory to support or exclude an MS diagnosis [24]. Disability was measured using EDSS scores [22]. Disease progression was assessed using the progression index (PI). This index was calculated by dividing the degree of disability, as measured using the EDSS, by the duration of the disease; the typical PI value is 0.4-0.6 [25]. We used the upper end of this range as a cut-off point to dichotomize MS patients into two groups: $PI > 0.6$ (severe progression) and $PI \leq 0.6$ (low progression).

Two experienced and trained researchers recorded the clinical and epidemiological characteristics of the participants, which included age, sex, treatment, duration of MS, and PI.

2.5. Genotyping CYP2R1 rs10766197 and CYP27B1 rs10877012 Polymorphisms. Peripheral whole blood samples, harvested in Vacutainer tubes containing ethylenediaminetetraacetic acid as an anticoagulant, were collected from each patient. Genomic DNA was obtained from these blood samples using the modified Miller technique [26]. Genomic DNA was quantified using a Nanodrop Genomic, and the DNA was diluted in Tris-EDTA buffer to 20 ng/μL and placed in 200 μL propylene cryotubes (Eppendorf™). Genotyping of *CYP2R1* rs10766197 and *CYP27B1* rs10877012 polymorphisms was performed by quantitative polymerase chain reaction (qPCR) using TaqMan probes [27]. TaqMan Assay IDs C_2958435_10 and C_26237740_10 were performed according to the manufacturer's instructions (Applied Biosystems); the StepOne™, Real-Time polymerase chain reaction (qPCR) system was employed for this purpose (Applied Biosystems). All results were independently scored by two investigators blinded to patient information. In case of ambiguous results, the sample was analysed a second time.

2.6. Quantification of Serum 25(OH) Vitamin D Levels. A peripheral venous blood sample was taken from each patient at the time of inclusion. Serum levels of 25(OH) vitamin D were quantified by ELISA using commercial kits (My BioSource; MBS580159, San Diego, CA, USA). All procedures were performed according to the manufacturer's recommendations. All measurements were performed by the same researchers, who were blinded to the clinical characteristics of the patients to avoid measurement biases.

2.7. Statistical Analysis. Qualitative variables were expressed as frequencies and percentages. Quantitative variables are expressed as the median, minimum (min), and maximum (max).

We identified genotypic frequencies by direct counting. Allelic frequencies were determined by direct counting of the observed genotypic frequencies. The chi-square test (or Fisher's exact test if required) was used for the comparison of proportions. For variables that did not satisfy the assumptions of parametric tests, Mann–Whitney U nonparametric tests were performed.

Odds ratio (OR) and the 95% confidence interval (95% CI) were calculated for genetic characteristics. The Kruskal-Wallis test was performed to compare serum 25(OH) vitamin D levels between genotypes. We also performed post hoc tests with the Bonferroni correction for multiple comparisons.

Hardy-Weinberg equilibrium (HWE) for CYP2R1 rs10766197 and CYP27B1 rs10877012 polymorphisms was evaluated in the groups and was determined by comparing the observed and expected data using the chi-squared test.

We performed a multivariable logistic regression analysis to identify variables associated with the MS. This model was adjusted by confunders (age and gender) and genotypes for genes. For the *CYP2R1 gene* rs10766197 GG was stated as referent and GA + AA was considered as risk. For the *CYP27B1* gene rs10877012 GG was used as referent and GT + TT was selected as risk. The variables included in the model were selected using a stepwise method.

A p value was considered significant at the $p \leq 0.05$ level. Data were analysed with R 4.1.0 base [28] and with the epiR [29] and ggplot2 packages [30].

2.8. Ethics and Consent. This study was approved by the Ethics Committee and Biosafety Committee from Centro Universitario de Ciencias de la Salud (CUCS) Universidad de Guadalajara, Jalisco, Mexico, with registration number CI-03519. This study was conducted according to the principles expressed in the Declaration of Helsinki. All study participants voluntarily provided written informed consent.

3. Results

We assessed 116 patients with MS and 226 individuals in the control group. The genotype distributions of the SNPs rs10766197 and rs10877012 in the control group were consistent with HWE (rs10766197 $p > 0.05$ and rs10877012 $p > 0.05$) (data not shown in tables).

The baseline characteristics of the MS patients are shown in Table 1. Of all MS patients included, 65.5% were females.

Table 1: Sociodemographic and clinical descriptive characteristics in multiple sclerosis patients.

Variable	Multiple sclerosis $n = 116$
Female, *n* (%)	76 (65.5)
Age, (years)	38 (19-66)
Disease characteristics	
Disease evolution, (years)	7.5 (1.0-28.0)
EDSS, (score)	3.0 (0.0-7.0)
Progression index, (score)	0.38 (0.0-4.5)
Severe progression (IP > 0.6), *n* (%)	39 (33.6)
Low progression (IP ≤ 0.6), *n* (%)	77 (66.4)
Serum 25(OH) vitamin D levels, (ng/mL)	18.3 (3.8-50.9)
Sufficient (>30 ng/mL), *n* (%)	14 (12.0)
Insufficient (20-30 ng/mL), *n* (%)	17 (14.7)
Deficient (<20 ng/mL), *n* (%)	85 (73.3)
Treatment	
Glatiramer acetate, *n* (%)	39 (33.6)
Interferon, *n* (%)	27 (23.3)
Rituximab, *n* (%)	12 (10.3)
Fingolimod, *n* (%)	11 (9.5)
Dimethyl fumarate, *n* (%)	7 (6.0)
Azathioprine, *n* (%)	5 (4.3)
Natalizumab, *n* (%)	4 (3.5)
No treatment, *n* (%)	11 (9.5)

EDSS: Expanded Disability Status Scale of Kurtzke for Multiple Sclerosis; IP: index progression (EDSS/disease evolution). Qualitative variables were expressed as frequency and percentage. Quantitative variables were expressed as median and min-max.

We observed a median age of 38 (19-66) years and a median of 7.5 (1-28) years of disease evolution. Overall, 33.6% of the patients with MS presented severe disease progression (PI > 0.6), while 66.4% presented low progression of the disease (IP < 0.6). Deficiency of serum 25(OH) vitamin D levels in 73.3% of MS patients was observed. The most prescribed treatment in MS was glatiramer acetate (33.6%), followed by interferons (23.3%) and rituximab (10.3%).

Table 2 presents the comparison of sociodemographic, serological, and genetic characteristics between MS patients and the control group. For rs10766197 of the *CYP2R1* gene, we observed a higher frequency of the heterozygous genotype GA (50.0%) in MS patients, while in the control group, we observed an increased frequency of the wild-type homozygous genotype GG (49.6%); however, these differences were not statistically significant ($p = 0.08$). The A allele was observed at a higher frequency in MS patients than in the control group (37.9% vs. 30.5%, $p = 0.05$). For rs10877012 of the *CYP2B1* gene, we observed a similar frequency of genotypes in both groups. We did not identify a significant difference between MS patients and the control group ($p = 0.90$). Additionally, in a subgroup of 106 individuals in the control group, we compared the 25(OH) vitamin D levels. Higher levels of 25(OH) vitamin D were observed in the control group than in the MS group ($p = 0.009$) (Figure 1). We identified a higher percentage of individuals who had sufficient 25(OH) vitamin D levels in the control group (43.4%, $p = 0.003$) and a higher percentage of individuals deficient in vitamin D in MS patients (73.3%, $p < 0.001$).

Table 3 shows the comparison of clinical characteristics between severe progression versus low progression in MS patients. We observed the same proportion of females in the severe progression and low progression groups (64.1% vs. 65.1%, respectively). We observed a shorter disease duration in the severe progression group than in the low progression group (3 years vs. 12 years, $p < 0.001$). In quantitative serum 25(OH) vitamin D levels, we did not observe a significant difference between the severe and low progression groups. In a stratified comparison of serum 25 (OH) vitamin D levels, we observed a higher percentage of individuals with deficient levels (<20 ng/mL) and found a significant difference between groups ($p = 0.037$). No differences were observed in genotypes and allele frequencies of either polymorphism between patients with severe vs. low disease progression.

Table 4 compares the genotypic and allelic frequencies of polymorphisms rs10766197 and rs10877012 between MS patients and the control group. In a comparison of the GG, GA, and AA genotypes of the rs10766197 polymorphism of *CYP2R1* between MS patients and the control group, a trend to almost significant differences were observed ($p = 0.08$). In a comparison of the GG, GT, and TT genotypes of the rs10877012 polymorphism of *CYP2B1* between MS patients and the control group, no differences were observed. OR and their 95% CI were calculated for the comparison between dominant and recessive genetic models. We observed a high risk of MS in the dominant model of rs10766197 of the *CYP2R1* gene (OR = 1.67; 95%CI = 1.05 – 2.64; $p = 0.02$). No differences in the risk of other genetic models or in rs10877012 of the *CYP2B1* gene polymorphism were observed.

Distribution analysis of serum 25(OH) vitamin D levels according to the genotypes of polymorphisms stratified between MS patients and the control group revealed that patients who carried the genotype GG for rs10766197 presented higher levels, with a median of 17.44 ng/mL (ranges of 8.45-45.32 ng/mL).

In data not shown, we performed a multivariable logistic regression analysis. After adjusting by confounders variables, we found that age (OR = 0.95, CI 95% 0.92-0.98; $p = 0.001$) and gender ratio (OR = 2.18, CI 95% 1.16-4.2; $p = 0.017$) were associated with an increase of risk for MS. The GA + AA genotypes of *CYP2R1* rs10766197 polymorphism were associated with an increase of MS (OR = 1.87, CI 95% 1.07-3.29; $p = 0.02$). However, the *CYP27B1* rs10877012 polymorphism was not associated with the disease.

In the comparison of serum 25(OH) vitamin D levels between GG, GA, and AA genotypes, we observed significant differences ($p = 0.046$), and in the post hoc analysis, a notably significant difference was observed between the GA and AA genotypes ($p = 0.029$). In the comparison of serum 25(OH) vitamin D levels between genotypes of rs10877012 of the *CYP2B1* gene, we did not observe a significant difference ($p = 0.94$) (Figure 2) (data not shown in tables).

In the comparison of genotypic and allelic frequencies of polymorphisms rs10766197 of the *CYP2R1* gene and rs10877012 of the *CYP27B1* gene between severe

Table 2: Comparison of sociodemographic, serological, and genetic characteristics of multiple sclerosis patients versus control group.

Variable	Multiple sclerosis $n = 116$	Control group $n = 226$	p
Female, n (%)	76 (65.5)	185 (81.9)	0.001
Age (years)	38 (19-66)	46.0 (21-66)	<0.001
Serum levels			
25(OH) vitamin D levels (ng/mL), n (%)	16.2 (3.8-50.9)	26.8 (4.3 – 53.2) *	0.009
Sufficient (>30 ng/mL), n (%)	14 (12.0)	46 (43.4) *	
Insufficient (20-30 ng/mL), n (%)	17 (14.7)	14 (13.2) *	0.009
Deficient (<20 ng/mL), n (%)	85 (73.3)	46 (43.4) *	
Genetic characteristics			
rs10766197, *CYP2R1 gene*			
Genotypes			
GG, n (%)	43 (37.1)	112 (49.6)	
GA, n (%)	58 (50.0)	90 (39.8)	0.08
AA, n (%)	15 (12.9)	24 (10.6)	
Allele $2n = 684$	$2n = 232$	$2n = 452$	
A, n (%)	88 (37.9)	138 (30.5)	0.05
G, n (%)	144 (62.1)	314 (69.5)	0.05
rs10877012, *CYP2B1 gene*			
Genotypes			
GG, n (%)	55 (47.4)	101 (44.7)	
GT, n (%)	48 (41.4)	98 (43.4)	0.90
TT, n (%)	13 (11.2)	27 (11.9)	
Allele $2n = 684$	$2n = 232$	$2n = 452$	
G, n (%)	158 (68.1)	300 (66.4)	0.65
T, n (%)	74 (31.9)	152 (33.6)	0.65

For rs10766197 of *CYP2R1 gene*: GG: wild homozygous; GA: heterozygous; AA: polymorphic homozygous. For rs10877012 of *CYP2B1 gene*: GG: wild homozygous; GT: heterozygous; TT: polymorphic homozygous. Qualitative variables were expressed as frequency and percentage. Quantitative variables were expressed as median and min-max. Comparisons between medians were performed using Mann–Whitney U test. Comparison between proportions was performed using Chi-square test (or Fisher exact test if applicable). p values were obtained comparing multiple sclerosis versus control group. *Serum levels of 25(OH) vitamin D were determined in 107 healthy controls.

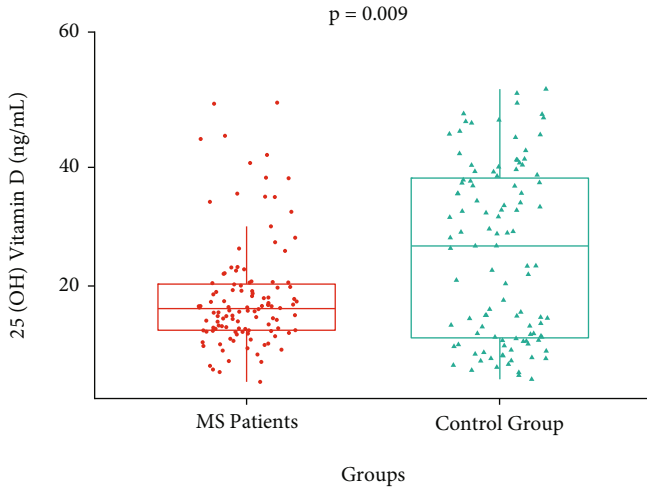


Figure 1: Levels of 25(OH) vitamin D in serum between MS patients and the control group ($p = 0.009$). The comparison was performed by Mann–Whitney U test.

progression vs. low progression in MS patients, we observed a similar frequency of the AA genotype for rs10766197 of the *CYP2R1* gene (15.4% in severe progression vs. 11.6% in low progression). Furthermore, for rs10877012 of *CYP2B1* gene, we observed a higher frequency in of the heterozygous GT genotype in the severe group (48.4%) than low progression group, in which we observed the wild homozygous GG with high frequency (51.9%). No significant differences were observed in the comparison of genotypes between the severe and low MS progression groups (data not shown in tables).

4. Discussion

In this study, we observed that the rs10766197 polymorphism of the *CYP2R1* gene confers a risk of MS. However, the rs10877012 polymorphism of the *CYP27B1* gene was not associated with the risk of MS. We report the first allelic and genotypic frequencies in a Latin American country for the rs10766197 polymorphism of the *CYP2R1* gene and the rs10877012 polymorphism of the *CYP27B1* gene.

Table 3: Comparison of clinical and genetic characteristics between severe progression and low progression in multiple sclerosis patients.

Variable	Severe progression (IP > 0.6) $n = 39$	Low progression (IP ≤ 0.6) $n = 77$	p
Female, n (%)	25 (64.1)	51 (66.2)	0.98
Age, (years)	36 (19-59)	40 (19-66)	0.13
Disease characteristics			
Disease evolution, (years)	3 (1-9)	12 (2-28)	<0.001
EDSS, (score)	4.0 (1-7)	2.5 (0-7)	0.008
Serum 25(OH) vitamin D levels, (ng/mL)	15.7 (3.8-50.9)	16.4 (5.4-45.3)	0.88
Sufficient (>30 ng/mL), n (%)	4 (10.3)	13 (16.9)	
Insufficient (20-30 ng/mL), n (%)	12 (30.7)	9 (11.7)	0.037
Deficient (<20 ng/mL), n (%)	23 (59.0)	55 (71.4)	
Treatment			
Glatiramer acetate, n (%)	9 (23.1)	30 (39.0)	0.10
Interferon, n (%)	7 (17.9)	20 (25.9)	0.36
Rituximab, n (%)	9 (23.1)	3 (3.9)	0.003
Fingolimod, n (%)	3 (7.7)	8 (10.4)	0.75
Dimethyl fumarate, n (%)	4 (10.3)	3 (3.9)	0.22
Azathioprine, n (%)	3 (7.7)	2 (2.6)	0.33
Natalizumab, n (%)	1 (2.5)	3 (3.9)	1.00
No treatment, n (%)	3 (7.7)	8 (10.4)	0.49
Genetic characteristics			
rs10766197, *CYP2R1 gene*			
Genotypes			
GG, n (%)	12 (30.8)	31 (40.3)	
GA, n (%)	21 (53.8)	37 (48.0)	0.58
AA, n (%)	6 (15.4)	9 (11.7)	
Allele $2n = 232$	$2n = 78$	$2n = 154$	
A, n (%)	33 (42.3)	55 (35.7)	0.33
G, n (%)	45 (57.7)	99 (64.3)	0.33
rs10877012, *CYP2B1 gene*			
Genotypes			
GG, n (%)	15 (38.5)	40 (51.9)	
GT, n (%)	19 (48.7)	29 (37.7)	0.39
TT, n (%)	5 (12.8)	8 (10.4)	
Allele $2n = 232$	$2n = 78$	$2n = 154$	
T, n (%)	49 (62.8)	45 (29.2)	<0.001
G, n (%)	29 (37.2)	109 (70.8)	<0.001

EDSS: Expanded Disability Status Scale of Kurtzke for Multiple Sclerosis; IP: index progression (EDSS/disease evolution). For rs10766197 of *CYP2R1 gene*: GG: wild homozygous; GA: heterozygous; AA: polymorphic homozygous. For rs10877012 of *CYP2B1 gene*: GG: wild homozygous; GT: heterozygous; TT: polymorphic homozygous. Qualitative variables were expressed as frequency and percentage. Quantitative variables were expressed as median and min-max. Comparisons between medians were performed using Mann–Whitney U test. Comparison between proportions was performed using Chi-square test (or Fisher exact test if applicable). p values were obtained comparing multiple sclerosis with severe progression versus multiple sclerosis with low progression.

CYP2R1 is responsible for the hydroxylation of vitamin D to 25(OH) vitamin D in the first activation step. It is thought to be an important determinant of the vitamin D metabolic pathway, as it shows a high affinity for vitamin D [31]. The SNP rs10766197 is located in the promoter region of the *CYP2R1* gene, which causes differences in gene expression [32]. The association between the rs10766197 polymorphism of the *CYP2R1* gene and MS has been previously identified. Scazzone et al. (2017) reported that an association between the GA genotype (heterozygous minor allele carriers) in Italian patients was 46% in MS patients vs. 43% in controls (OR 2.19, 95% CI 1.09-4.39, $p = 0.03$), and the frequency of the AA genotype (homozygous minor allele carriers) was 39% in MS patients vs. 25% in controls (OR 3.18, 95% CI 1.52-6.65, p 5 0.002) [19]. Other studies analysed other polymorphisms in this gene. Laursen et al. found

Table 4: Comparison of genotypic and allelic frequencies of polymorphisms rs10766197 of *CYP2R1 gene* and rs10877012 of *CYP2B1 gene*, between multiple sclerosis patients and control group.

	Multiple sclerosis $n = 116$	Control group $n = 226$	OR	95% CI	p
rs10766197, *CYP2R1 gene*					
Genotypes					
GG $n = 155$ (%)	43 (37.1)	112 (49.6)	—	—	
GA $n = 140$ (%)	58 (50.0)	90 (39.8)	—	—	0.08
AA $n = 39$ (%)	15 (12.9)	24 (10.6)	—	—	
GA + AA versus GG (as referent)	73 (62.9)	114 (50.4)	1.67	1.05-2.64	0.03
GA + GG versus AA (as referent)	101 (87.0)	202 (89.4)	0.8	0.40-1.59	0.59
Alleles 2n =684	*2n =232*	*2n =452*			
A allele, 2n =226 (%)	88 (37.9)	138 (30.5)	1.39	0.99-1.94	0.05
G allele, $2n = 458$ (%)	144 (62.1)	314 (69.5)	0.72	0.52-1.00	0.05
rs10877012, *CYP2B1 gene*					
Genotypes					
GG $n = 156$ (%)	55 (47.4)	101 (44.7)	—	—	
GT $n = 146$ (%)	48 (41.4)	98 (43.4)	—	—	0.90
TT $n = 40$ (%)	13 (11.2)	27 (11.9)	—	—	
GT + TT versus GG (as referent)	61 (52.6)	125 (55.3)	0.89	0.57-1.40	0.63
GG + GT versus TT (as referent)	103 (88.8)	199 (88.1)	1.08	0.53-2.17	0.84
Allele $2n = 684$	$2n = 232$	$2n = 452$			
G allele, $2n = 458$ (%)	158 (68.1)	300 (66.4)	1.08	0.77-1.52	0.65
T allele, $2n = 226$ (%)	74 (31.9)	152 (33.6)	0.92	0.66-1.30	0.65

For rs10766197 of *CYP2R1 gene*: GG: wild homozygous; GA: heterozygous; AA: polymorphic homozygous; for rs10877012 of *CYP2B1* gene: GG: wild homozygous; GT: heterozygous; TT: polymorphic homozygous. OR: odds ratio risk; 95% CI: 95% confidence interval. p values were obtained comparing multiple sclerosis versus control group.

a significant association of CYP2R1 rs10741657 with MS risk in a cross-sectional study performed on the Danish population [33].

In our study, we found that the frequency of vitamin D deficiency was lower in MS patients than in controls. Our findings are supported by other studies; Oliveira et al., 2017 reported an association between serum levels of 25(OH) vitamin D and MS, reporting and media of MS patients (26.12 ± 8.47 ng/mL) vs. controls (29.71 ± 9.17 ng/mL, $p = 0.02$) [34], and Munger et al., 2006 found that the risk of MS decreased with increasing serum levels of 25(OH) vitamin D [35]. Several hypotheses have been proposed in advance to explain the role of vitamin D in the MS. First, vitamin D influences the balance of CD4 T lymphocytes, by decreasing Th1 and Th17 cell differentiation and promoting Th2 and regulatory T cells (Tregs) proliferation. In the development of MS, CD4 T lymphocytes (or T helper lymphocytes, including Th1, Th2, Th17 subsets) play a crucial role in the activation of myelin-specific Th1 and Th17 cells, which drives the inflammation within the central nervous system [36, 37]. Second, MS is characterized by progressive demyelination, vitamin D is deemed to have a role in the myelination and remyelination processes [38–41], and finally, 80% of MS-associated genes are enriched for VDREs in their promoter region and consequently, and they are regulated by vitamin D [42].

CYP27B1, encoding 1a-hydroxylase, which converts 25(OH) vitamin D to its active form, 1,25(OH)2D, is the cytochrome P450 gene most strongly associated with vitamin D status [15]. The SNP rs10877012 located on the 5′ untranslated region (UTR) may affect transcript stabilization and the posttranscriptional control of mRNA [43]. For the rs10877012 polymorphism of the *CYP27B1* gene, we observed that genotype or allele does not confer a clinically relevant risk for the development of MS. These results are in agreement with those reported by Simon et al. in American women with MS; in the study, findings do not support a role for an independent effect of the vitamin D-related gene polymorphisms investigated and the risk of MS [44]. The authors also identified three additional SNPs on the *CYP27B1* gene associated with MS [19].

Genome-wide association studies (GWAS) and other epidemiological studies have shown that SNPs within the *CYP2R1* gene modulate 25(OH) vitamin D levels [45–47]. In our study, we found that MS patients carrying one or two A alleles had lower 25(OH) vitamin D serum levels in comparison to patients carrying allele G. Scazzone et al. reported the rs10766197 distribution in MS patients, stratified according to the 25(OH) vitamin D concentrations, and the patients who carried the AA genotype presented a trend of lower levels of 25(OH) vitamin D in comparison to those with a genotype of GG or GA, although the difference was not statistically significant [14]. In contrast, other

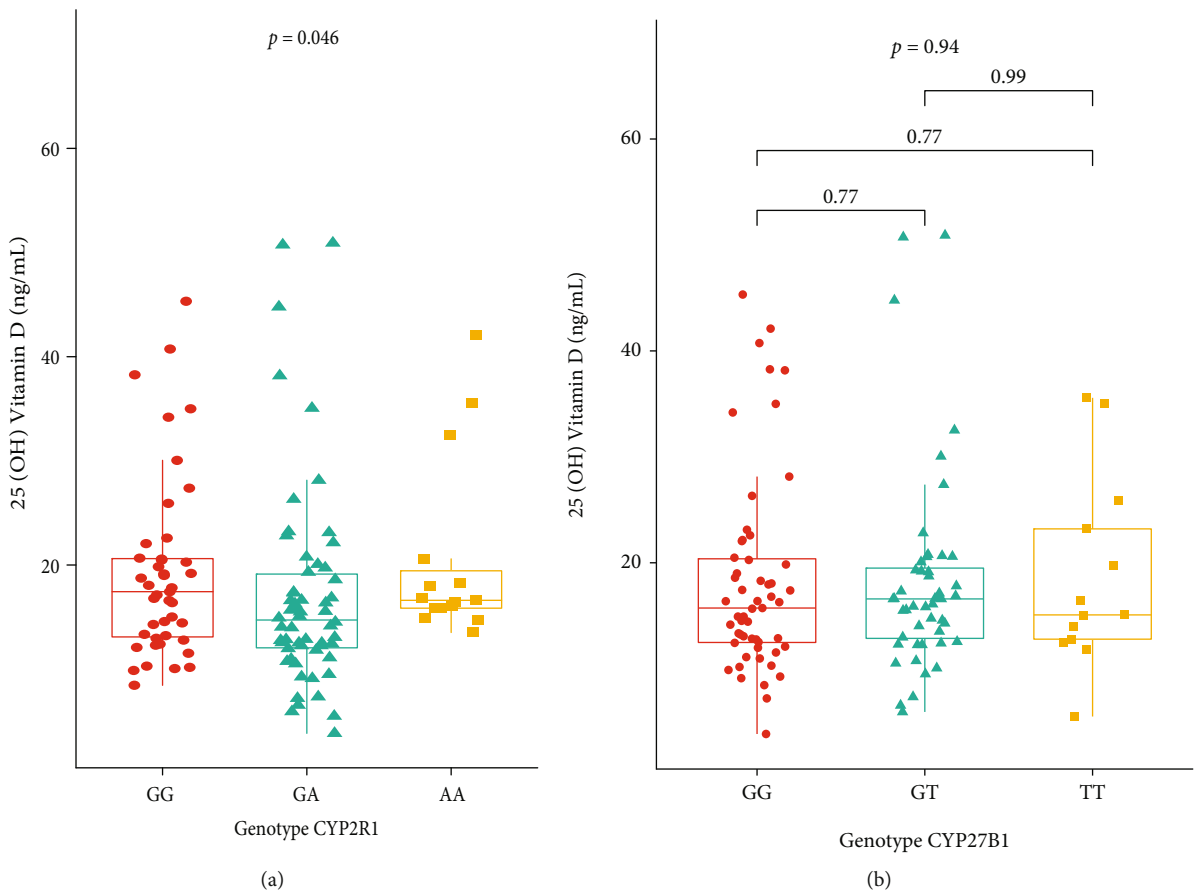


Figure 2: Comparison of levels of 25(OH) vitamin D in serum according to genotypes of single nucleotide polymorphisms rs10766197 of CYP2R1 gene and rs10877012 of CYP27B1 gene between MS patients. (a) Comparison of the levels of 25(OH) vitamin D in serum between genotypes of rs10766197 of the CYP2R1 gene. (b) Comparison of the levels of 25(OH) vitamin D in serum between genotypes of rs10877012 of the CYP27B1 gene. Data are presented as medians with ranges (min-max) of 25(OH) vitamin D (ng/mL). The comparison between genotype groups was designed by the Kruskal-Wallis test and Bonferroni correction to perform multiple comparisons.

studies have reported an association of the rs10766197 polymorphism with the highest mean levels of 25(OH) vitamin D in the healthy Chinese population [48].

We did not find an association between the rs10877012 polymorphism of the *CYP27B1* gene and 25(OH) vitamin D levels. However, this association has been reported in other diseases. Ramos-Lopez et al. reported that the rs10877012 C allele was associated with lower levels of 25(OH) vitamin D in a study of patients with gestational diabetes [20].

There could be several reasons why we were not able to find an association between the SNPs and 25(OH) vitamin D levels. These inconsistent results could be derived from differences in population selection and ethnicity. The serum 25(OH) vitamin D levels of the population we studied were much lower than those in other published reports [49–51]. ENSANUT in 2006 reported that approximately 30% of adults aged 20 to 60 years have hypovitaminosis D [51].

It has been reported in Mexico that 37% of women of reproductive age have deficient levels and 50% have insufficient levels of vitamin D [52].

To date, to the best of our knowledge, this is the first study to analyse the association between these two polymorphisms, 25(OH) vitamin D levels and MS, in Mexican patients, which enabled us to examine the interactions between the *CYP2R1* gene, *CYPB27* gene, and vitamin D levels to assess how these synergisms may influence MS risk.

There are limitations to the present investigation. First, in relation to the findings of the main effects of individual SNPs and MS risk, these genes were not submitted to an exhaustive examination. However, we cannot exclude the possibility that other gene regions may be important. Second, we did not measure 25-hydroxylase activity. Third, our study was limited to a small sample size.

MS is a multifactorial disease, and this finding supports the notion that risk factors may be relevant only in a proportion of the population with underlying genetic susceptibility [53]. Future investigations that could replicate the findings in this work are necessary to verify the biological precept of the plausibility of gene-environment interactions as they relate to vitamin D and MS risk.

5. Conclusion

Reduced serum 25(OH) vitamin D levels were observed in MS patients, although these levels were not associated with disease progression. Carriers of GA + AA rs10766197 of

the *CYP2R1* gene had an increased risk of MS. None of these polymorphisms was associated with severe progression on MS disease.

Data Availability

The database used to support the findings of this study is available on request. If this database is required, please direct the correspondence to Dr. Ana M. Saldaña-Cruz (ana .saldanac@academicos.udg.mx).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Martinez-Hernandez A contributed to the investigation, writing, original draft, review and editing, and final approval. Perez-Guerrero EE contributed to the methodology, investigation, resources, writing, original draft, review and editing, formal analysis, and final approval. Macias-Islas MA contributed to the methodology, investigation, resources, writing, original draft, review and editing, and final approval. Nava-Valdivia CA contributed to the methodology, investigation, formal analysis, writing, original draft, review and editing, and final approval. Villagomez-Vega A contributed to the investigation, original draft, review and editing, and final approval. Contreras-Haro B contributed to the writing original draft, review and editing, and final approval. Garcia-Ortega YE contributed to the investigation, writing, original draft, review and editing, and final approval. Esparza-Guerrero Y contributed to the investigation, writing, original draft, review and editing, and final approval. Gallardo-Moya SG contributed to the investigation, original draft, review and editing, and final approval. Gamez-Nava JI contributed to the investigation, original draft, and final approval. Gonzalez-Lopez contributed to the original draft, and final approval. Oliva-Flores E contributed to the investigation, original draft, and final approval. Rodriguez-Jimenez NA contributed to the investigation, original draft, and final approval. Cortes-Enriquez F contributed to the investigation, original draft, and final approval. Saldaña-Cruz AM contributed to the conceptualization, methodology, investigation, resources, funding acquisition, supervision, project administration, original draft, writing, review and editing, and final approval.

Acknowledgments

Martinez-Hernandez A, Esparza-Guerrero Y and Gallardo-Moya SG received scholarships from Consejo Nacional de Ciencia y Tecnologıa (Mexico). This project was supported by a grant proportionate for the University of Guadalajara: Fondo para Proyectos de Impulso a la Investigación (PIN 2020).

References

[1] A. Compston and A. Coles, "Multiple sclerosis," *The Lancet*, vol. 372, no. 9648, pp. 1502–1517, 2008.

[2] A. J. Thompson, S. E. Baranzini, J. Geurts, B. Hemmer, and O. Ciccarelli, "Multiple sclerosis," *The Lancet*, vol. 391, no. 10130, pp. 1622–1636, 2018.

[3] Y. Rito, J. Flores, A. Fernández-Aguilar et al., "Vitamin D and disability in relapsing–remitting multiple sclerosis in patients with a Mexican background," *Acta Neurologica Belgica*, vol. 118, no. 1, pp. 47–52, 2018.

[4] F. D. Lublin, S. C. Reingold, J. A. Cohen et al., "Defining the clinical course of multiple sclerosis: the 2013 revisions," *Neurology*, vol. 83, no. 3, pp. 278–286, 2014.

[5] D. Karussis, "The diagnosis of multiple sclerosis and the various related demyelinating syndromes: a critical review," *Journal of Autoimmunity*, vol. 48-49, pp. 134–142, 2014.

[6] M. Macías Islas and E. Ciampi, "Assessment and impact of cognitive impairment in multiple sclerosis: an overview," *Biomedicines*, vol. 7, no. 1, p. 22, 2019.

[7] N. Ghasemi, S. Razavi, and E. Nikzad, "Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy citation: Ghasemi N, Razavi Sh, Nikzad E. Multiple sclerosis: pathogenesis, symptoms, diagnoses, and cell-based therapy," *Cell Journal (Yakhteh)*, vol. 19, no. 1, pp. 1–10, 2017.

[8] A. Ascherio, K. L. Munger, R. White et al., "Vitamin D as an early predictor of multiple sclerosis activity and progression," *JAMA Neurology*, vol. 71, no. 3, pp. 306–314, 2014.

[9] T. J. Littlejohns, W. E. Henley, I. A. Lang et al., "Vitamin D and the risk of dementia and Alzheimer disease," *Neurology*, vol. 83, no. 10, pp. 920–928, 2014.

[10] K. L. Munger, J. Åivo, K. Hongell, M. Soilu-Hänninen, H. M. Surcel, and A. Ascherio, "Vitamin D status during pregnancy and risk of multiple sclerosis in offspring of women in the Finnish Maternity Cohort," *JAMA Neurology*, vol. 73, no. 5, pp. 515–519, 2016.

[11] E. A. Dziedzic, J. S. Gasior, M. Pawłowski, and M. Dabrowski, "Association of vitamin D deficiency and degree of coronary artery disease in cardiac patients with type 2 diabetes," *Journal of Diabetes Research*, vol. 2017, Article ID 3929075, 11 pages, 2017.

[12] J. M. Lemire, J. S. Adams, R. Sakai, and S. C. Jordan, "1 alpha,25-Dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells," *The Journal of clinical investigation*, vol. 74, no. 2, pp. 657–661, 1984.

[13] S. Chen, G. P. Sims, X. X. Chen, Y. Y. Gu, S. Chen, and P. E. Lipsky, "Modulatory effects of 1,25-dihydroxyvitamin D 3 on human B cell differentiation," *The Journal of Immunology*, vol. 179, no. 3, pp. 1634–1647, 2007.

[14] C. Scazzone, L. Agnello, G. Bivona, B. Lo Sasso, and M. Ciaccio, "Vitamin D and genetic susceptibility to multiple sclerosis," *Biochemical Genetics*, vol. 59, no. 1, pp. 1–30, 2021.

[15] J. B. Cheng, M. A. Levine, N. H. Bell, D. J. Mangelsdorf, and D. W. Russell, "Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase," *Proceedings of the National Academy of Sciences*, vol. 101, no. 20, pp. 7711–7715, 2004.

[16] E. Axen, H. Postlind, H. Sjoberg, and K. Wikvall, "Liver mitochondrial cytochrome P450 CYP27 and recombinant-expressed human CYP27 catalyze 1 alpha-hydroxylation of

25-hydroxyvitamin D3," *Biochemistry*, vol. 91, no. 21, pp. 10014–10018, 1994.

[17] T. Shinki, H. Shimada, S. Wakino et al., "Cloning and expression of rat 25-hydroxyvitamin D3-1 -hydroxylase cDNA," *Biochemistry*, vol. 94, no. 24, pp. 12920–12925, 1997.

[18] A. R. Baker, D. P. Mcdonnellt, M. Hughest, T. M. Crisp, D. J. Mangelsdorft, M. R. Haussler et al., *Biochemistry §Present address*, vol. 85, Garvan Institute of Medical Research, Saint Vincents Hospital, New South, 1988.

[19] L. Agnello, C. Scazzone, B. Lo Sasso et al., "VDBP, CYP27B1, and 25-hydroxyvitamin D gene polymorphism analyses in a group of Sicilian multiple sclerosis patients," *Biochemical Genetics*, vol. 55, no. 2, pp. 183–192, 2017.

[20] E. Ramos-Lopez, H. Kahles, S. Weber et al., "Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms," *Diabetes, Obesity and Metabolism*, vol. 10, no. 8, pp. 683–685, 2008.

[21] S. C. Sánchez, "Mestizaje y características físicas de la población mexicana," *Arqueología Mexicana*, vol. 65, pp. 64–67, 1996.

[22] J. F. Kurtzke, "On the origin of EDSS," *Multiple Sclerosis and Related Disorders*, vol. 4, no. 2, pp. 95–103, 2015.

[23] R. Bakshi, A. J. Thompson, M. A. Rocca et al., "MRI in multiple sclerosis: current status and future prospects," *The Lancet Neurology*, vol. 7, no. 7, pp. 615–625, 2008.

[24] G. Arrambide, M. Tintore, C. Espejo et al., "The value of oligoclonal bands in the multiple sclerosis diagnostic criteria," *Brain*, vol. 141, no. 4, pp. 1075–1084, 2018.

[25] W. S. Cendrowski, "Progression index and disability status in multiple sclerosis: a resurvey of 207 patients in central Poland," *Schweizer Archiv für Neurologie und Psychiatrie*, vol. 137, no. 4, pp. 5–13, 1986.

[26] S. MWer, D. Dykes, and H. Polesky, "A Simple Salting out Procedure for Extracting DNA from Human Nucleated Cells," *Nucleic Acids Research*, vol. 15, 1987.

[27] K. J. Livak, "Allelic discrimination using fluorogenic probes and the 5′ nuclease assay," *Genetic Analysis*, vol. 14, no. 5-6, pp. 143–149, 1999.

[28] R. Core, *Team. A Language and Environment for Statistical Computing*, RC Team, 2021.

[29] M. Stevenson, T. Nunes, C. Heuer et al., *epiR: Tools for the Analysis of Epidemiological Data*, R package version 2.0.38, 2021.

[30] H. Wickham, *ggplot2: Elegant Graphics for Data Analysis*, Springer-Verlag, New York, 2016.

[31] R. Shinkyo, T. Sakaki, M. Kamakura, M. Ohta, and K. Inouye, "Metabolism of vitamin D by human microsomal CYP2R1," *Biochemical and Biophysical Research Communications*, vol. 324, no. 1, pp. 451–457, 2004.

[32] M. Wjst, J. Altmüller, T. Faus-Kessler, C. Braig, M. Bahnweg, and E. André, "Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signalling pathway," *Respiratory Research*, vol. 7, no. 1, p. 7, 2006.

[33] J. H. Laursen, H. B. Søndergaard, A. Albrechtsen et al., "Genetic and environmental determinants of 25-hydroxyvitamin D levels in multiple sclerosis," *Multiple Sclerosis Journal*, vol. 21, no. 11, pp. 1414–1422, 2015.

[34] S. R. Oliveira, A. N. C. Simão, D. F. Alfieri et al., "Vitamin D deficiency is associated with disability and disease progression in multiple sclerosis patients independently of oxidative and nitrosative stress," *Journal of the Neurological Sciences*, vol. 381, no. 381, pp. 213–219, 2017.

[35] K. L. Munger, L. I. Levin, B. W. Hollis, N. S. Howard, and A. Ascherio, "Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis," *Jama*, vol. 296, no. 23, p. 2832, 2006.

[36] J. M. Fletcher, S. J. Lalor, C. M. Sweeney, N. Tubridy, and K. H. Mills, "T cells in multiple sclerosis and experimental autoimmune encephalomyelitis," *Clinical and Experimental Immunology*, vol. 162, no. 1, pp. 1–11, 2010.

[37] L. Legroux and N. Arbour, "Multiple sclerosis and T lymphocytes: an entangled story," *Journal of Neuroimmune Pharmacology*, vol. 10, no. 4, pp. 528–546, 2015.

[38] M. Goudarzvand, M. Javan, J. Mirnajafi-Zadeh, S. Mozafari, and T. Tiraihi, "Vitamins E and D3 attenuate demyelination and potentiate remyelination processes of hippocampal formation of rats following local injection of ethidium bromide," *Cellular and molecular neurobiology*, vol. 30, no. 2, pp. 289–299, 2010.

[39] F. Chiuso-Minicucci, L. L. W. Ishikawa, L. A. N. Mimura et al., "Treatment with vitamin D/MOG association suppresses experimental autoimmune encephalomyelitis," *PLoS One*, vol. 10, no. 5, article e0125836, 2015.

[40] L. A. N. Mimura, F. Chiuso-Minicucci, T. F. C. Fraga-Silva et al., "Association of myelin peptide with vitamin D prevents autoimmune encephalomyelitis development," *Neuroscience*, vol. 317, no. 317, pp. 130–140, 2016.

[41] J. Matías-Guíu, C. Oreja-Guevara, J. A. Matias-Guiu, and U. Gomez-Pinedo, "Vitamina D y remielinizacion en la esclerosis multiple," *Neurología (English edition)*, vol. 33, no. 3, pp. 177–186, 2018.

[42] S. V. Ramagopalan, D. A. Dyment, M. Z. Cader et al., "Rare variants in the CYP27B1 gene are associated with multiple sclerosis," *Annals of Neurology*, vol. 70, no. 6, pp. 881–886, 2011.

[43] S. Yu, Y. Feng, C. Qu et al., "_CYP27B1_ as an instrument gene to investigate the causal relationship between vitamin D deficiency and obesity: a family-based study," *European Journal of Clinical Nutrition*, vol. 74, no. 5, pp. 806–810, 2020.

[44] K. C. Simon, K. L. Munger, Xing Yang, and A. Ascherio, "Polymorphisms in vitamin D metabolism related genes and risk of multiple sclerosis," *Multiple Sclerosis*, vol. 16, no. 2, pp. 133–138, 2010.

[45] J. Ahn, K. Yu, R. Stolzenberg-Solomon et al., "Genome-wide association study of circulating vitamin D levels," *Human Molecular Genetics*, vol. 19, no. 13, pp. 2739–2745, 2010.

[46] T. J. Wang, F. Zhang, J. B. Richards et al., "Common genetic determinants of vitamin D insufficiency: a genome-wide association study," *The Lancet*, vol. 376, no. 9736, pp. 180–188, 2010.

[47] A. Arabi, N. Khoueiry-Zgheib, Z. Awada et al., "CYP2R1 polymorphisms are important modulators of circulating 25-hydroxyvitamin D levels in elderly females with vitamin insufficiency, but not of the response to vitamin D supplementation," *Osteoporosis International*, vol. 28, no. 1, pp. 279–290, 2017.

[48] Z. Zhang, J. W. He, W. Z. Fu, C. Q. Zhang, and Z. L. Zhang, "An analysis of the association between the vitamin D pathway and serum 25-hydroxyvitamin D levels in a healthy Chinese population," *Journal of Bone and Mineral Research*, vol. 28, no. 8, pp. 1784–1792, 2013.

[49] R. Jorde, M. Witham, W. Janssens et al., "Vitamin D supplementation did not prevent influenza-like illness as diagnosed retrospectively by questionnaires in subjects participating in randomized clinical trials," *Scandinavian Journal of Infectious Diseases*, vol. 44, no. 2, pp. 126–132, 2012.

[50] S. I. Hassanein, M. A. Abu El Maaty, H. M. Sleem, and M. Z. Gad, "Triangular relationship between single nucleotide polymorphisms in the CYP2R1 gene (rs10741657 and rs12794714), 25-hydroxyvitamin d levels, and coronary artery disease incidence," *Biomarkers*, vol. 19, no. 6, pp. 488–492, 2014.

[51] M. Flores, L. M. Sánchez-Romero, N. Macías, A. Lozada, E. Díaz, and S. Barquera, *Concentraciones séricas de vitamina D en niños, adolescentes y adultos mexicanos*, Resultados de la ENSANUT, 2006.

[52] A. Contreras-Manzano, S. Villalpando, and R. Robledo-Pérez, "Vitamin D status by sociodemographic factors and body mass index in Mexican women at reproductive age," *Salud Pública de México*, vol. 59, no. 5, pp. 518–525, 2017.

[53] A. I. Ruiz-Ballesteros, M. R. Meza-Meza, B. Vizmanos-Lamotte, I. Parra-Rojas, and U. de la Cruz-Mosso, "Association of vitamin D metabolism gene polymorphisms with autoimmunity: evidence in population genetic studies," *International Journal of Molecular Sciences*, vol. 21, no. 24, pp. 9626–9724, 2020.

Hindawi
Journal of Immunology Research
Volume 2021, Article ID 9782994, 15 pages
https://doi.org/10.1155/2021/9782994

Review Article

Role of Vitamin D in Systemic Sclerosis: A Systematic Literature Review

Alexandra-Diana Diaconu,[1] **Iustina Ostafie**,[1] **Alexandr Ceasovschih**,[2,3] **Victorița Șorodoc**,[2,3] **Cătălina Lionte**,[2,3] **Codrina Ancuța**,[1,3] **and Laurențiu Șorodoc**[2,3]

[1]*2nd Rheumatology Department, Clinical Rehabilitation Hospital, 700661 Iasi, Romania*
[2]*2nd Internal Medicine Department, "Sf. Spiridon" Emergency Clinic County Hospital, 700111 Iasi, Romania*
[3]*Faculty of Medicine, University of Medicine and Pharmacy "Grigore T. Popa", 700115 Iasi, Romania*

Correspondence should be addressed to Alexandr Ceasovschih; alexandr.ceasovschih@yahoo.com and Codrina Ancuța; codrina_ancuta@yahoo.com

Received 6 August 2021; Accepted 29 October 2021; Published 29 November 2021

Academic Editor: Vlad Padureanu

Background. Systemic sclerosis (SSc) is a chronic multisystem autoimmune condition defined by a complex pathobiology, comprising excessive fibrosis of skin and internal organs, peripheral vasculopathy with endothelial cell dysfunction, inadequate vascular repair and neovascularization, and aberrant immunity. Vitamin D is a steroid hormone with pleiotropic effects beyond its traditional role in calcium and bone homeostasis. Since vitamin D has immunomodulatory, cardioprotective, and antifibrotic properties, it could potentially interfere with SSc pathogenesis. Suboptimal vitamin D levels are classically recognized in scleroderma, irrespective of clinical and serological phenotype. *Aim*. This systematic review is aimed at investigating and clarifying the role of vitamin D in SSc and emphasizing the association of vitamin D status with different clinical settings. *Methods and Results*. A systematic online search was performed, using PubMed databases to collect articles on the topic of vitamin D in SSc. The final analysis included 40 eligible articles. *Conclusions*. Hypovitaminosis D is common in SSc patients and could be associated with clinical and serologic patterns of the disease. Intervention for low serum vitamin D levels in SSc pathogenesis remains controversial, as well as the significance of vitamin D supplementation in such patients.

1. Introduction

1.1. Systemic Sclerosis. Classified as an orphan disease, systemic sclerosis (SSc) remains a chronic multisystem autoimmune condition driven by a multifaceted link between excessive fibrosis of the skin and internal organs, peripheral vasculopathy with endothelial cell dysfunction succeeded by inadequate vascular repair and neovascularization, and aberrant immunity. The clinical picture ranges from peripheral vasculopathy (Raynaud's phenomenon, digital ulcers, and critical digital ischemia) and skin involvement, to a broad spectrum of manifestations depending on the presence and degree of internal organ (gastrointestinal, lung, kidney, and heart) involvement, resulting in specific phenotypes characterized by precise antibodies, distinct prognoses, and specific management [1, 2].

Although the complex pathobiology of SSc is still not well understood, immunological, vascular, and fibrotic abnormalities remain key events, including (i) fibroproliferative vascular injuries of small arteries and arterioles; (ii) increased production of profibrotic growth factors such as transformer-β growth factor (TGF-β), connective tissue growth factor, and insulin-like growth factor, leading to fibrosis of the skin and various internal organs; and (iii) various modifications of innate, humoral, and cellular immunity, promoting immune cell trafficking into the affected tissues and

autoantibody synthesis. Additionally, the intervention of environmental factors in a genetically susceptible host triggers the autoimmune mechanism specific to SSc [2, 3].

1.2. Vitamin D. Vitamin D is a steroid hormone obtained from endogenous release in the skin following sunlight exposure and from food intake. Calcitriol or vitamin D_3 is actually formed by the activation of a precursor on skin exposed to ultraviolet B radiation and, to become active, requires two metabolic conversions: 25-α hydroxylation in the liver and 1-α hydroxylation in the kidney [4].

Apart from its traditional role in maintaining calcium homeostasis and bone health [5], vitamin D has pleiotropic effects based on the ubiquitous distribution of vitamin D receptors (VDRs). Belonging to the nuclear hormone receptors superfamily [6], VDRs are able to mediate the multifaceted biological effects of hormone D, as they are found not only in the osteoblasts, small intestine, and kidneys but also in a variety of immune cells (such as activated T and B lymphocytes, mononuclear cells, antigen-presenting cells, and natural killer cells), islet beta cells, and in some organs (such as the heart, brain, skin, breasts, gonads, and prostate) [5]. Beyond the classic antirachitic role, vitamin D has protective effects in many dermatological, cardiovascular, gastrointestinal, pulmonary, renal, and autoimmune disorders [7].

Among the physiological vitamin D actions, the regulation of cell differentiation, proliferation, apoptosis, angiogenesis, innate or adaptive immunity, and immune modulation are of particular interest in immune-mediated rheumatic disorders. Vitamin D mainly exerts its immunosuppressive effects by inhibiting T helper-1 (Th1) lymphocytes and proinflammatory cytokines (IL-6 and IL-17) and stimulating anti-inflammatory cytokine production (IL-4 and IL-10). Furthermore, it is able to switch the Th1 response (IL-1, TNF-α, and IFN-γ) into a Th2 one (autoantibodies and TGF-β). Vitamin D also hampers the Th17 response and stimulates regulatory T (Treg) cells. Therefore, vitamin D seems to be protective in the development of autoimmunity, related to its immunomodulatory and tolerance effects [7].

Interestingly, vitamin D has antifibrotic properties, being able to modulate TGF-β signaling and inhibit the profibrotic phenotype of skin and lung fibroblasts. In fact, vitamin D downregulates the expression of profibrotic factors such as TGF-β1 and collagen I and III, while upregulating several antifibrotic factors [7].

Moreover, the role of vitamin D in cardioprotection is widely accepted, although its mechanisms are still poorly understood. It seems that vitamin D acts via the renin system to decrease blood pressure, increase vascular endothelial growth factor, and decrease the production of endothelium-derived contracting factors [7].

The Institute of Medicine (Washington DC) has proposed cut-off values for normal plasma vitamin D levels (>30 ng/ml), vitamin D insufficiency (21–30 ng/ml), and deficiency (<20 ng/ml) [8]. While vitamin D deficiency results in impaired phospho-calcium homeostasis and promotes low bone mineral density and osteoporosis [9], poor vitamin D status may also trigger various autoimmune conditions [7].

1.3. Vitamin D in Systemic Sclerosis. In the last decades, many authors have focused on vitamin D status in systemic sclerosis. Due to its immunomodulatory, cardioprotective, and antifibrotic biological effects, vitamin D could interfere with each of the pathobiological mechanisms activated in SSc, including autoimmunity, peripheral vasculopathy, and fibrosis. A majority of studies have emphasized suboptimal vitamin D levels in SSc patients, irrespective of the clinical or serological phenotype. It seems that vitamin D levels are significantly lower compared to healthy controls, and vitamin D supplementation at the usual dose fails to correct the deficit. Furthermore, due to the extreme clinical heterogeneity of the disease, poor vitamin D status in SSc may or may not be associated with different systemic manifestations of the disease, SSc activity and severity, or disease subtype [7].

Despite extensive research on vitamin D in autoimmune conditions, the potential role of hypovitaminosis D in the pathobiology of scleroderma, the dual relationship between serum vitamin D levels and different clinical manifestations, and the importance of vitamin D supplementation within the clinical and serologic spectrum of SSc remain controversial.

This systematic literature review is aimed at investigating the pathobiological role of vitamin D in systemic sclerosis and emphasizing the association of vitamin D status with different clinical settings.

2. Methods

A systematic online search was performed using the PubMed databases up to 31 May 2021 to collect articles on vitamin D in systemic sclerosis, without any criteria based on the year, language, or type of publication. We used the following combinations of terms: "systemic sclerosis" and "vitamin D", "scleroderma" and "vitamin D", and "systemic scleroderma" and "vitamin D".

All articles were retrieved and assessed independently by two reviewers, who highlighted data regarding the authors, publication date, and characteristics of the study. After rejecting abstracts and duplicates, all articles in full text were examined to verify their eligibility criteria, which included studies on the association between vitamin D and SSc. The exclusion criteria were preprint papers, articles with no relevant content to the purpose of the research, reviews, studies with a pediatric population, *in vitro* studies or experimental models, and studies with a small group of patients (case series and case reports).

3. Results

We identified 428 publications focusing on vitamin D in SSc in the PubMed databases. The final analysis included 40 eligible articles after 388 were excluded (59 abstracts, 175 duplicates, 154 irrelevant, *in vitro* experimental models on the vitamin D effects or its analogues, case reports, non-English, or pediatric population articles). These data are illustrated in Figure 1.

Study design, number of cases, and main outcomes of selected articles are summarized in Table 1. The majority

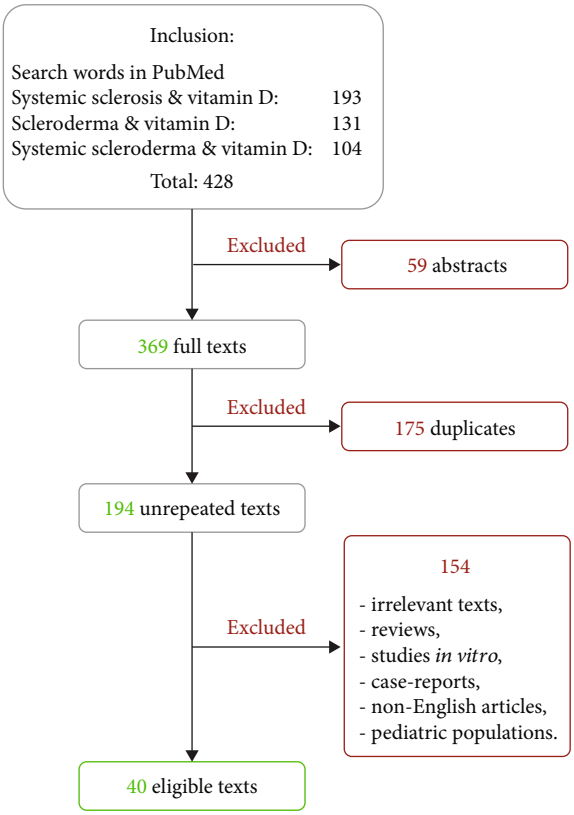


Figure 1: Flowchart of study selection.

of studies focused on assessing vitamin D levels in SSc and the relationship between vitamin D and different disease subtypes and clinical and serologic scenarios, while only a few articles focused on potential pathobiological relationships or the role of vitamin D supplementation.

4. Discussions

4.1. Hypovitaminosis D in SSc. Vitamin D deficiency has been implicated in many degenerative, metabolic, inflammatory, and autoimmune rheumatic conditions, including systemic sclerosis. The prevalence of hypovitaminosis D in SSc remains a topic of interest, and several papers have aimed to investigate the association between serum vitamin D levels and different aspects of SSc. Overall, irrespective of the clinical and serological disease settings, SSc patients present with lower levels of vitamin D compared with healthy controls [12, 14, 15, 19, 20, 23–26, 31, 35, 37, 44, 45]. Although hypovitaminosis D is frequently reported, some authors have found significantly lower levels ($p < 0.001$) and vitamin D deficiency [15, 19, 20, 25, 31, 35, 37], while others have found only insufficient levels. A closer look at the literature confirmed lower vitamin D levels in patients with SSc, independent of the well-known age, gender, and seasonal variations, as well as related to comorbidities, drugs, and life habits [14, 15, 19, 20, 23–26, 31, 35, 37, 40, 41, 45].

Although seasonal variations in vitamin D levels are predictable, with peak values during summer [22], Seriolo et al. found lower average serum 25(OH)D concentrations in all seasons in SSc patients [42].

Additionally, in a retrospective cohort study, Arnson et al. found a negative correlation between vitamin D concentrations and age ($p < 0.05$) [40], while many other studies were unable to demonstrate any correlation between serum vitamin D and age, gender, body mass index (BMI), and therapy in SSc [20, 22, 26, 29, 32, 33, 41, 42]. Moreover, there is no association between vitamin D serum levels and the duration and frequency of sun exposure or sunblock use in SSc [22].

Conversely, Sampaio-Barros et al. demonstrated a positive correlation between vitamin D levels and weight and BMI [32], and Arnson et al. found a negative correlation between vitamin D concentration and patient age in SSc [40]. Only one study [46] emphasized the association between hypovitaminosis D and ethnicity (Arab origin) in SSc.

Furthermore, the relationship between disease duration and hypovitaminosis D also appears to be variable, probably because of the heterogeneity of the populations investigated. In a study of 65 SSc patients, Caramaschi et al. found an association between vitamin D deficiency and longer disease duration.

Although the role of vitamin D in the development of different clinical and serological phenotypes of SSc remains controversial, it has been suggested that hypovitaminosis D may have a role in disease activity and/or severity. However, this hypothesis was not validated in our review; the majority of authors demonstrated no association between vitamin D levels and disease activity [16, 19, 21, 22, 33, 34, 46] or severity (Medsger disease severity scores) [6, 33]. Only Vacca et al. emphasized a significant negative correlation between vitamin D and the European Disease Activity Score [6].

Another important point is related to the true origin of low-serum vitamin D in SSc. Poor vitamin D status in SSc could be multifactorial, since its normal metabolism typically comprises epidermal synthesis, gastrointestinal absorption, and hepatic and renal hydroxylation steps, and SSc is defined by various degrees of skin, gastrointestinal, and renal involvement. Though still controversial, potential explanations for either insufficient or deficient vitamin D include (i) impaired epidermal synthesis due to skin thickening and hyperpigmentation; (ii) insufficient intake or impaired gastrointestinal absorption due to gastrointestinal involvement and/or certain drugs (such as glucocorticoids or anticonvulsants for neuropathic pain) that could interfere with vitamin absorption; and (iii) limited sun exposure related to physical disability, depression, and potential psychological limitations [30]. Moreover, according to Carmel et al., the presence of vitamin D antibodies may also account for lower vitamin D levels in SSc, as they found anti-25(OH) vitamin D IgM antibodies more frequently in SSc cases compared to controls [33].

Some studies have focused on statistically significant relationships between vitamin D and parathyroid hormone (PTH), not only in healthy individuals but also in SSc

Table 1: Characteristics of selected studies on serum vitamin D in systemic sclerosis.

Reference	Study design	Patients	Outcome
Lo Gullo et al., 2021 [10]	Case-control study	36 SSc patients and 36 healthy controls	Correlation between serum vitamin D and CD34+ cell ($p = 0.05$). No correlation between serum vitamin D levels and CRP ($p = 0.451$). No difference in vitamin D levels in dcSSc patients compared to lcSSc patients. No association between vitamin D, body mass index, and endothelial markers in SSc.
Isola et al., 2021 [11]	Clinical trial	35 SSc patients, 36 with periodontitis (PD), 36 with both SSc and periodontitis, and 37 controls	Lower vitamin D values in subjects with PD and SSc plus PD than to SSc and healthy subjects ($p < 0.001$). Negative association between vitamin D levels and PD in SSc ($p = 0.011$). Association between lower vitamin D and CRP ($p < 0.01$).
Hax et al., 2020 [12]	Case-control study	50 SSc patients and 35 healthy nonmatched controls	Lower vitamin D levels in SSc patients ($p = 0.014$). 25-Hydroxyvitamin D [25(OH)D] levels inversely correlated with parathyroid hormone (PTH) levels ($p = 0.026$). No significant associations between vitamin D and serum cytokines. No association between vitamin D serum levels and the duration and frequency of sun exposure ($p = 0.417$ and $p = 0.295$, respectively) or with the sun block use ($p = 0.857$).
Paolino et al., 2020 [13]	Retrospective study	36 consecutive postmenopausal SSc female patients	Significant differences in malnourished SSc patients in the median values of PTH ($p = 0.02$) and vitamin D levels ($p = 0.008$).
González-Martín et al., 2020 [14]	Cross-sectional study	70 patients diagnosed with SSc (diffuse or limited forms)	Lower levels of vitamin D in 59% of the SSc patients. Inverse association between serum vitamin D levels and carotid intima-media thickness ($p = 0.025$).
Horváth et al., 2019 [15]	Case-control study	44 SSc patients and 33 healthy controls	Vitamin D deficiency in SSc patients ($p = 0.003$).
Caimmi et al., 2019 [16]	Retrospective longitudinal study	65 SSc patients	Negative association between vitamin D levels and the risk of digital ulcers developing ($p = 0.018$). No significant differences in vitamin D between SS with or without vitamin D supplementation ($p = 0.922$). No significant differences in vitamin D variations for disease subset ($p = 0.728$), disease activity ($p = 0.463$), previous digital ulcers ($p = 0.379$), incident pulmonary arterial hypertension ($p = 0.646$), delta of body mass index ($p = 0.824$), delta of forced vital capacity ($p = 0.633$) or diffusion capacity of carbon monoxide (DLCO) ($p = 0.647$), smoking habit ($p = 0.333$), modified Rodnan skin score (mRSS) ($p = 0.295$).
Di Liberto et al., 2019 [17]	Prospective case-control study	45 SSc patients and 35 controls	The treatment with 1,25(OH)(2)D of regulatory T cells increased the production of IL-10, a cytokine able to modulate immune response ($p < 0.0001$).

Table 1: Continued.

Reference	Study design	Patients	Outcome
Corallo et al., 2019 [18]	Prospective study	62 SSc Caucasian patients	No association between serum vitamin D levels and sarcopenia ($p = 0.3$).
Gupta et al., 2018 [19]	Pilot study	38 SSc patients, 38 controls	Significantly lower serum vitamin D levels in SSc patients compared with healthy controls ($p = 0.001$). No correlation between serum vitamin D levels and age, gender, disease duration or its variants, type of autoantibodies, presence of digital ulceration, or systemic involvement. Inverse correlation between serum vitamin D levels and mRSS ($r = -0.267$).
Taylan et al., 2018 [20]	Cross-sectional study	46 SSc patients and 30 healthy controls	Significantly lower vitamin D levels in SSc patients ($p < 0.05$).
Kotyla et al., 2018 [21]	Case-control study	48 patients with diffuse systemic sclerosis and 23 controls	A weak correlation between vitamin D levels and iFGF23 ($p < 0.05$). No association between vitamin D levels and the extent of skin involvement or disease activity ($p = 0.97$).
Trombetta et al., 2017 [22]	Retrospective study	154 SSc patients	A significant correlation of vitamin D levels with lung involvement ($p = 0.04$), peripheral vascular ($p = 0.03$), kidney ($p = 0.02$), and gastrointestinal damage ($p = 0.05$) and with seasonality ($p = 0.0086$) in SSc patients. Correlation between 25(OH)D and calcium serum concentrations ($p = 0.05$). No statistically significant correlation between 25(OH)D and gender ($p = 0.63$), age ($p = 0.81$), Raynaud's phenomenon duration ($p = 0.69$), disease duration ($p = 0.43$), dcSSc, lcSSc ($p = 0.49$). No significant correlations between digital ulcer incidence and 25(OH)D serum concentrations ($p = 0.13$).
Ahmadi et al., 2017 [23]	Case-control study	60 SSc patients (30 diffuse scleroderma and 30 limited scleroderma), 30 age- and sex-matched healthy controls	Lower serum levels of vitamin D in the SSc patients compared with healthy controls ($p < 0.001$). No significant differences in serum vitamin D levels between dcSSc and lcSSc ($p = 0.395$).
An et al., 2017 [24]	Meta-analysis	554 SSc patients and 321 controls	Lower serum vitamin D levels in SSc compared with healthy controls, in dcSSc compared to lcSSc. No correlation between vitamin D deficiency and mRSS ($p = 0.47$), systolic pulmonary pressure ($p = 0.72$), gastrointestinal ulcer ($p = 0.98$), and pulmonary involvement ($p = 0.99$).
Hajialilo et al., 2017 [25]	Cross-sectional study	60 SSc patients and 60 healthy controls	Significantly lower vitamin D levels in SSc patients ($p = 0.001$). No significant correlation between 25(OH)D levels and the presence of calcinosis and positive results for autoantibodies.
Zhang et al., 2017 [26]	Case-control study	60 SSc patients and 60 healthy controls	Lower vitamin D levels ($p < 0.001$) in SSc patients compared with healthy controls. High ratio of pulmonary involvement in patients with vitamin D insufficiency.

Table 1: Continued.

Reference	Study design	Patients	Outcome
Giuggioli et al., 2017 [27]	Case-control study	140 patients	Hypovitaminosis D associated with autoimmune thyroiditis ($p = 0.008$) and calcinosis ($p = 0.057$). Decreased 25(OH)D levels correlated with increased PTH ($p < 0.0001$).
Park et al., 2017 [28]	Pilot study	40 female SSc patients and 80 healthy controls	Vitamin D deficiency associated with digital ulcer ($p = 0.012$), but not with atherosclerosis or arterial stiffness ($p > 0.05$).
Cruz-Domínguez et al., 2017 [29]	Cohort study	220 SSc patients	Lower vitamin D levels in SSc with and without calcinosis. No association between vitamin D levels and calcinosis ($p = 0.56$).
Groseanu et al., 2016 [30]	Cross-sectional study	51 SSc patients	Positive correlation between decreased vitamin D levels and pulmonary fibrosis ($p = 0.011$) and low DLCO ($p = 0.019$). Negative correlation between vitamin D status and diastolic dysfunction ($p = 0.033$), digital contractures ($p = 0.036$), and muscle weakness ($p = 0.015$). Negative association between vitamin D supplementation and development of digital ulcers ($p = 0.04$).
Atteritano et al., 2016 [31]	Case-control study	40 patients with scleroderma and 40 healthy controls	Significantly lower serum vitamin D in SSc patients ($p = 0.0003$). Inverse correlation between vitamin D serum concentrations in SSc and systolic pulmonary artery pressure ($p = 0.013$). Significant correlation between vitamin D and PTH serum levels in SSc ($p < 0.05$). Significant association between vitamin D insufficiency and mRSS ($p = 0.02$).
Sampaio-Barros et al., 2016 [32]	Cross-sectional study	38 female patients with diffuse SSc	Lower levels of vitamin D in anti-Scl-70-positive compared to anti-Scl-70-negative SSc ($p = 0.039$). Positive correlation of vitamin D levels with weight ($p = 0.041$), BMI ($p = 0.038$), total femur BMD ($p = 0.037$), femoral neck BMD ($p = 0.017$), SF-36-Vitality ($p = 0.017$), SF-36-Social Function ($p = 0.05$), SF-36-Emotional Role ($p = 0.049$), and SF-36-Mental Health ($p = 0.0006$). Negative correlation between 25(OH)D and quality of life in dcSSc: HAQ-Reach ($p = 0.044$) and HAQ-Grip Strength ($p = 0.042$). Negative correlation between vitamin D levels and severe nailfold capillaroscopy alterations: diffuse devascularization ($p = 0.029$) and avascular areas ($p = 0.033$).
Carmel et al., 2015 [33]	Case-control study	54 SSc patients and 41 healthy controls	Positive correlation between IgM 25(OH)D antibodies and scleroderma ($p = 0.002$). No correlation between vitamin D antibodies and other autoantibodies, disease severity, or target organ damage.

Table 1: Continued.

Reference	Study design	Patients	Outcome
Corrado et al., 2015 [34]	Case-control study	64 postmenopausal SSc patients and 35 healthy control postmenopausal women	Significantly lower 25(OH)D levels in dcSSc compared to the lcSSc ($p < 0.001$). A significant association between degree skin fibrosis and circulating levels of 25(OH)D ($p < 0.05$). No correlation between 25(OH)D levels and presence of anti-centromere or anti-topoisomerase I autoantibodies and the disease duration. No correlation between malabsorption and 25(OH)D levels.
Atteritano et al., 2013 [35]	Case-control study	54 SSc women and 54 postmenopausal controls	Significantly lower vitamin D levels in SSc patients ($p < 0.001$). Significant correlation between vitamin D and PTH levels in SSc ($p < 0.001$).
Rios Fernández et al., 2012 [36]	Case-control study	100 SSc patients and 100 controls	Lower levels of vitamin D in SSc from the north of Spain in comparison with those in south of Spain ($p < 0.031$). Low bone mineral density (BMD) in 86% SSc with low levels of vitamin D (<30 ng/ml) compared with 66.7% of those with normal levels ($p = 0.073$). Significant association between vitamin D level heart involvement ($p = 0.012$), positive antinuclear antibody (ANA) ($p < 0.006$), and low DLCO ($p = 0.017$).
ibn Yacoub et al., 2012 [37]	Case-control study	60 SSc patients and 60 controls	Very low levels of vitamin D ($p = 0.001$) in SSc patients compared with controls. Significant correlation between vitamin D levels and joint pain severity, immunological status, and BMD in lumbar spine and femoral neck.
Avouac et al., 2012 [38]	Cross-sectional study	71 women with SSc, 139 women with RA, and 227 healthy women	Low levels of vitamin D is a risk factor for fractures ($p = 0.03$).
Shinjo et al., 2011 [39]	Case-control study	10 patients with JoSSc and 10 healthy controls	High prevalence of 25(OH)D insufficiency in JoSSc ($p = 0.04$) and correlation with hip BMD (femoral neck and total femur: $p = 0.004$ and $p = 0.02$, respectively).
Arnson et al., 2011 [40]	Retrospective cohort study	327 European patients with SSc and 141 healthy controls	Lower serum vitamin D concentrations ($p < 0.001$) and inverse relationship with cutaneous tissue fibrosis ($p = 0.02$). A negative correlation between vitamin D concentration and patient age ($p < 0.05$).
Gambichler et al., 2011 [41]	Observational study	137 SSc patients	Lower vitamin D levels ($p < 0.0001$). No significant relationship between serum 25(OH)D levels and SSc subtypes, lung fibrosis, renal involvement, gastroesophageal reflux disease, digital ulcers, mRSS, ANA, age, sex, BMD, and therapy.
Seriolo et al., 2011 [42]	Case-control study	53 SSc female patients with SSc and 35 sex-, age-, and season-matched control	During winter: vitamin D insufficiency in 60% SSc compared with 38% matched controls ($p < 0.001$); lower average serum 25(OH)D levels among SSc compared with controls ($p < 0.001$). During summer: 64% SSc patients and 36% controls with vitamin D insufficiency ($p < 0.001$); 24% SSc vs. 12% control with vitamin D deficiency ($p < 0.0001$); lower average serum 25(OH)D levels among SSc compared with controls ($p < 0.001$).

Table 1: Continued.

Reference	Study design	Patients	Outcome
Caramaschi et al., 2010 [43]	Prospective study	65 SSc patients	Association between patients with vitamin D deficiency and longer disease duration ($p = 0.026$), lower DLCO (0.014), higher estimated PAP ($p = 0.037$), higher values of erytrocyte sedimentation rate (ESR) ($p = 0.01$), and C-reactive protein (CRP) ($p = 0.004$) and with nailfold videocapillaroscopic pattern ($p = 0.013$) in comparison with patients with vitamin D insufficiency.
Rios Fernández et al., 2010 [44]	Cohort study	63 SSc patients	Lower vitamin D levels. No significant correlation between vitamin D levels and sPAP and the presence or absence of lung fibrosis ($p > 0.05$).
Calzolari et al., 2009 [45]	Case-control study	60 SSc patients and 60 matched controls	Lower levels of vitamin D in SSc patients compared with controls ($p < 0.001$). No significant association between vitamin D concentrations and disease features (lcSSc or dcSSc, gastrointestinal involvement, cutaneous ulcers, and joint involvement) and no correlation with mRSS. Vitamin D and serum C-telopeptide of type I collagen negatively correlated in SSc ($p = 0.01$). Vitamin D correlates with physical performance score assessed by the Medical Outcomes Study SF-36 (Short Form-36 questionnaire) ($p = 0.08$).
Vacca et al., 2009 [6]	Prospective study	156 consecutive SSc patients (90 from Northern France and 66 from Southern Italy)	Slight association between vitamin D and anti-centromere antibodies ($p = 0.04$). Significant negative correlation between low serum vitamin D levels and European Disease Activity Score ($p = 0.04$). No correlation with CRP ($p = 0.67$). Vitamin D deficiency associated with sPAP estimated by echocardiography ($p = 0.004$) and pulmonary fibrosis ($p = 0.04$). No associations between vitamin D deficiency and acroosteolysis, calcinosis, HAQ, or Medsger disease severity scores.
Braun-Moscovici et al., 2008 [46]	Retrospective study	134 Mediterranean SSc patients	Hypovitaminosis D significantly correlated with ethnicity (Arab origin) ($p = 0.009$). Statistically significant relationship between vitamin D and PTH ($p = 0.01$), but not between vitamin D and acroosteolysis ($p = 0.447$).
Hulshof et al., 2000 [47]	Double-blind, placebo-controlled trial	27 patients: 20 with morphea and 7 with SSc	No significant difference in SSc between the placebo and 1,25(OH)(2)D groups after 9 months of treatment in the skin score, esophageal body motility, and oral aperture. No significant change in S-PIIINP in serum samples of SSc patients after 1,25(OH)(2)D treatment.
Humbert el al., 1993 [48]	Open prospective, uncontrolled study	11 SSc patients	Vitamin D treatment improved significantly oral aperture and flexion index distance ($p < 0.05$) and skin extensibility ($p < 0.01$).

patients [12, 13, 31, 35, 46]. Furthermore, Hax et al. demonstrated that 25-hydroxyvitamin D [25(OH)D] levels are inversely correlated with PTH [12]. Paolino et al. identified significant differences in malnourished SSc patients in the median values of PTH and vitamin D levels [13].

4.2. Vitamin D Levels and SSc-Related Clinical and Serologic Manifestations

4.2.1. Skin Involvement and Clinical Phenotype. Almost always involved in systemic sclerosis, skin is a marker of disease activity, severity, and prognosis. The modified Rodnan skin score (mRSS) remains the most appropriate technique to evaluate the extent and severity of skin fibrosis [49]. Extensive and excessive skin thickening, as well as widespread skin fibrosis, correlates with severe internal organ involvement, high disability, and poor prognosis [49].

It has been suggested that typical skin manifestations such as fibrosis and, to a lesser extent, calcinosis cutis may reduce vitamin D synthesis and influence the other clinical manifestations in scleroderma patients, with differences between diffuse (dcSSc) and limited cutaneous SSc (lcSSc). A closer look at the potential association between levels of vitamin D and the clinical phenotype of SSc showed no significant difference in vitamin D or its active metabolite 25(OH)D levels, irrespective of the disease subtype, in the majority of studies [10, 16, 22–24, 41, 45]. However, An et al. demonstrated lower serum vitamin D in SSc compared with healthy controls and in dcSSc compared to lcSSc. Further, Corrado et al. found significantly lower 25(OH)D levels in dcSSc compared to lcSSc.

Lower serum vitamin D inversely correlates with the extent of cutaneous fibrosis, as reported by two studies [31, 40]. In their retrospective cohort study including 327 SSc patients, Arnson et al. demonstrated an inverse correlation between skin fibrosis and vitamin D levels [40]. Additionally, Atteritano et al. showed higher mRSS in SSc with vitamin D insufficiency [31]. On the other hand, several papers failed to report associations between vitamin D concentrations and the extent of skin involvement or mRSS [16, 21, 41, 48].

It is widely recognized that vitamin D also has antifibrotic properties related to its essential intervention in fibrogenesis through the modulation of TGF-β activity. Moreover, the inhibitory effects of vitamin D on collagen and hyaluronate production induced by TGF-β have been extensively recognized. TGF-β remains a key factor involved in collagen synthesis by fibroblasts, and VDR expression is a negative regulator of the TGF-β, a signaling pathway. The inhibition of the profibrotic TGF-β signaling via vitamin D is enhanced by its role in polarizing the local immune response. In addition, it seems that aberrant VDR expression in fibroblasts in SSc patients is responsible for the profibrotic effects of TGF-β on fibroblasts via decreased vitamin D concentrations [20]. This is why lower levels of vitamin D in SSc patients may be related to skin fibrosis, and the correction of hypovitaminosis D could interfere with fibrosis.

Interestingly, Humbert et al. described a significantly improved skin flexibility after vitamin D supplementation [48] but, more recently, Hulshof et al. failed to recognize any significant variation in mRSS after 9 months of vitamin D supplementation in their double-blind placebo-controlled trial [47].

Finally, regarding the association between vitamin D and calcinosis cutis (an SSc cutaneous manifestation commonly found in CREST syndrome), the majority of studies described no significant correlation between 25(OH)D levels and the presence of calcinosis in patients with SSc [6, 25, 27, 29, 41].

4.2.2. Peripheral Digital Vasculopathy. Different studies have investigated the association between low vitamin D levels and peripheral vasculopathy in SSc patients, particularly Raynaud's phenomenon and digital ulcers. Although this is a nonspecific but widely reported vasospastic condition in the majority of patients with scleroderma [50, 51], it seems that neither the severity nor the duration of Raynaud's phenomenon correlates with vitamin D levels [22]. SSc patients with low vitamin D have an increased risk of developing digital ulcers, as supported by multiple studies [16, 28], and vitamin D deficiency may be an independent risk factor for digital ulcers in such patients. Conversely, other papers failed to demonstrate a significant relationship between serum 25(OH)D and digital ulcers [19, 22, 41, 45]. Moreover, in a cross-sectional study, Groseanu et al. pointed out a negative association between vitamin D supplementation and the development of digital ulcers in patients with SSc [30].

Nailfold videocapilaroscopy is largely used to assess microvascular changes in SSc, and specific capillaroscopic patterns not only reflect the diagnosis but also predict internal organ involvement and prognosis [52]. Various different papers have addressed the potential association between vitamin D and capillaroscopic features [32, 43]. Sampaio-Barros et al. clearly showed that diffuse devascularization and avascular areas depicted on videocapillaroscopy are associated with lower vitamin D concentrations in scleroderma patients [32]. Additionally, Caramaschi et al. found an association between the late nailfold videocapillaroscopy pattern and vitamin D deficiency [43].

Extreme clinical heterogeneity of SSc is closely related to a broad spectrum of internal organ involvement comprising, but not limited to, gastrointestinal, cardiovascular, pulmonary, and renal injuries (Figure 2) [2]. Moreover, SSc can be fatal, with a 3-year survival rate in half of the cases, especially with severe lung or heart involvement [53].

Hypovitaminosis D is a general finding in scleroderma [3–5, 9, 10, 13, 15, 16, 21, 25, 27, 34, 35], and based on its pleiotropic effects, including its immunomodulatory, cardioprotective, and antifibrotic properties, low vitamin D status could influence the pathogenetic pathways activated in SSc [30]. Nevertheless, the association between abnormal vitamin D status and SSc onset, or any of its clinical manifestations, is still under debate [30]. Gupta et al. did not observe any significant association between vitamin D levels and systemic involvement [19]. Conversely, several other studies found that patients with a poor vitamin D status presented with systemic features, particularly lung and cardiac involvement [6, 22, 26, 30, 31, 36, 43].

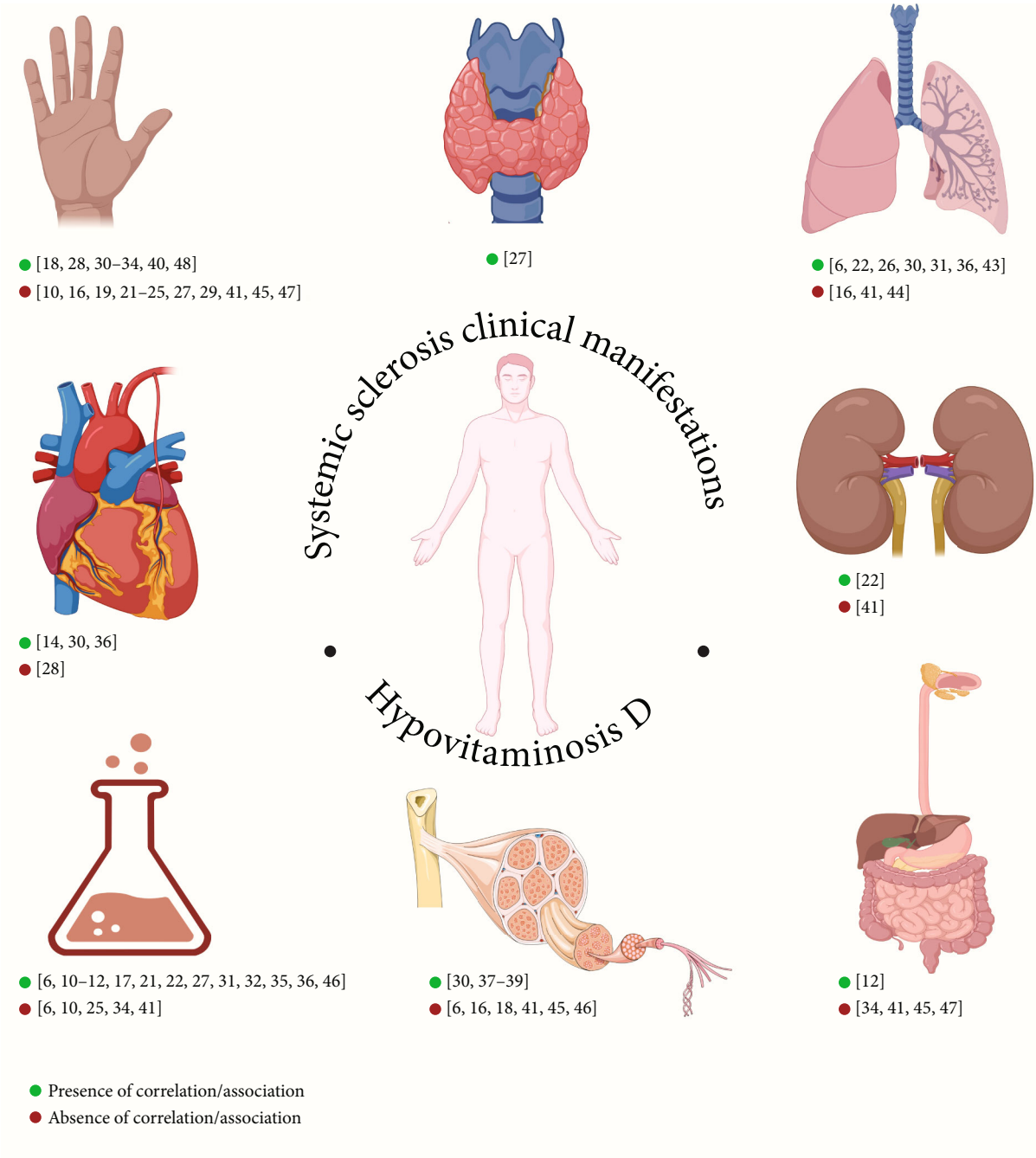


Figure 2: Relationship between systemic sclerosis clinical manifestations and hypovitaminosis D.

4.2.3. Pulmonary Involvement. Interstitial lung disease and pulmonary arterial hypertension are the most frequent subtypes of lung involvement (over 80% of cases). Trombetta et al., in a 152 SSc patient retrospective analysis, and Zhang et al., in a case-control study including 120 participants, found a higher rate of pulmonary involvement in patients with vitamin D deficiency [22, 26]. However, data regarding the potential association of vitamin D and pulmonary manifestations in SSc remain controversial. Thus, although Atteritano et al., Caramaschi et al., and Vacca et al. all pointed out a significant correlation between pulmonary hypertension and lower levels of vitamin D [6, 31, 43], Caimmi et al. and Rios Fernández et al. did not find any significant difference between the frequency of pulmonary arterial hypertension and vitamin D concentrations [16, 44]. The exact mechanism and pathways through which suboptimal levels of vitamin D may be responsible for pulmonary hypertension also remain unclear [20]. Potential explanations include the activation of

the renin-angiotensin pathway, aberrant expression of prostacyclin by vascular smooth muscle cells, or secondary parathyroidism [30].

It has been suggested that low vitamin D levels may be a factor associated with certain clinical manifestations, especially pulmonary and cardiac involvement in SSc, promoting disease activity and severity. Indeed, hypovitaminosis D significantly correlates with lung involvement [6, 22, 26, 30, 36, 43], with a high ratio of more severe pulmonary fibrosis in SSc patients with vitamin D insufficiency, irrespective of disease subset [26, 30]. A positive correlation between decreased serum vitamin D and pulmonary fibrosis, as well as low diffusion capacity of carbon monoxide (DLCO), has been demonstrated [6, 30, 36]. Moreover, Groseanu et al. confirmed that none of the patients with normal vitamin D status developed lung fibrosis in their study, and lung involvement was reported more frequently in patients with a diffuse form and vitamin D deficiency [30]. On the other hand, Caimmi et al. found an absence of significant differences in vitamin D variations for delta of forced vital capacity, or DLCO, in SSc [16], and An et al. found no correlation between vitamin D deficiency and systolic pulmonary pressure and pulmonary involvement [24]. Rios Fernández et al. and Gambichler et al. failed to support any significant correlation between vitamin D levels and the presence or absence of lung fibrosis [41, 44].

As mentioned previously, vitamin D also acts as a factor influencing the transdifferentiation of lung epithelial cells into myofibroblasts in murine studies, supporting the direct role of vitamin D in fibrosis signaling. This may suggest the potential benefits of using vitamin D as an adjuvant for immunosuppression in patients with clinical findings where fibrosis predominates, such as in lung and skin involvement [30].

4.2.4. Cardiac Involvement. The cardioprotective effect of vitamin D is widely accepted, and significant correlations between vitamin D status and cardiovascular involvement have been reported in scleroderma patients. Since cardiovascular involvement is commonly seen during SSc with repeated focal ischemic lesions, fibroblast replacement, and subsequent irreversible myocardial fibrosis as the main causes of myocardial impact, many authors have been interested in assessing the role of vitamin D deficiency in cardiovascular involvement secondary to SSc. Groseanu et al. were able to demonstrate that patients with SSc and vitamin D deficiency had a higher prevalence of systolic or diastolic dysfunction, as well as rhythm and conduction disorders [30]. Rios Fernández et al. reported an association between vitamin D levels and heart involvement [36].

Vitamin D may also have several effects on microvascular and macrovascular involvement in patients with SSc. Different studies have aimed to clarify the controversial topic of subclinical atherosclerosis in SSc patients by assessing carotid intima-media thickness (cIMT), flow-mediated vasodilatation, and coronary and cerebral calcifications. In a recent study, González-Martín et al. reported an inverse association between serum vitamin D levels and cIMT in SSc [14]. On the other hand, in a pilot study including 120 participants, no differences between the median cIMT, brachial-ankle pulse wave velocity, frequency of carotid plaques, and vitamin D deficiency were demonstrated [28]. Further studies are necessary in order to clarify the association between hypovitaminosis D and micro- and macrovascular involvement in patients with SSc.

4.2.5. Gastrointestinal Tract Involvement. Gastrointestinal involvement has been widely reported in SSc [54, 55], mainly related to vasculopathy and local fibrosis [56] and may potentially prompt vitamin D malabsorption. However, there is no consensus on low vitamin D levels and gastrointestinal manifestations in SSc. The majority of studies have failed to demonstrate any association between gastrointestinal manifestations and suboptimal vitamin D status. Calzolari et al. found no relationship between gastrointestinal involvement and vitamin D concentrations [45]. Moreover, Gambichler et al. documented no association between gastroesophageal reflux disease and vitamin D [41], while Hulshof et al. also revealed no significant differences in esophageal motility after 9 months of vitamin D supplementation [47]. Small intestinal dysmotility caused by atrophy or fibrosis in the intestinal wall seems to be quite common in patients with SSc and may be complicated by bacterial overgrowth and malnutrition [55]. However, Corrado et al. found no correlation between malabsorption and vitamin D levels [34]. No difference in body mass index values depending on the level of vitamin D was demonstrated either [16, 41].

4.2.6. Renal Involvement. Renal diseases in SSc patients may manifest in different clinicopathological settings, and scleroderma renal crisis is the most severe life-threatening complication [57, 58]. In a 154 patient retrospective analysis, Trombetta et al. found a high ratio of kidney involvement at lower vitamin D levels [22], and Groseanu et al. reported scleroderma renal crisis more frequently in patients with vitamin D deficiency in their cohort with SSc [30]. Conversely, Gambichler et al. did not find any significant relationship between renal involvement and hypovitaminosis D, irrespective of SSc subtype [41].

4.2.7. Musculoskeletal Involvement. Musculoskeletal involvement is a common and potentially disabling manifestation in scleroderma patients [30]. Since vitamin D is essential for proper functioning of the musculoskeletal system, several studies have investigated the role of suboptimal vitamin D levels and articular involvement, acroosteolysis, sarcopenia, and myositis during SSc.

While Ibn Yacoub et al. reported a significant correlation between vitamin D levels and severity of joint pain [37], another study showed no significant statistical association between vitamin D and joint involvement [45]. According to Groseanu et al., patients with vitamin D deficiency developed synovitis more frequently than those with vitamin D insufficiency. A negative correlation was also identified between vitamin D status and muscle weakness; patients with muscle weakness presented with lower vitamin D levels compared to those without [30].

Defined by the European Working Group on Sarcopenia in Older People as a progressive loss of muscle mass and strength, with a risk of adverse outcomes such as disability, poor quality of life, and death [59], sarcopenia may develop in different immune-mediated rheumatic conditions due to muscle loss secondary to abnormal proinflammatory status, pain, decreased activity, and glucocorticoid use. Corallo et al. evaluated the presence of sarcopenia in a cohort of 62 SSc patients using two specific parameters, Relative Skeletal Mass Index and Hand Grip Strength. No association between serum vitamin D levels and sarcopenia was found, but the authors highlighted age-related sarcopenia [18].

Although not pathognomonic, but rather highly suggestive for SSc, acroosteolysis is a distinct pattern of bone resorption which affects the distal phalanges, due to repeated vascular injury. Hyperparathyroidism secondary to low vitamin D might also contribute to acroosteolysis, but we found two studies that showed no correlation between vitamin D and acroosteolysis [6, 46].

Many recent articles have tried to clarify the association of low vitamin D levels with bone mineral density (BMD) in SSc and fracture risk in SSc patients, based on the role of vitamin D in calcium-phosphate and bone homeostasis. Overall, decreased bone mineral density (BMD) was reported in the majority of scleroderma patients presenting with low levels of vitamin D (<30 ng/ml) [36–39]. In addition, Shinjo et al. identified a high prevalence of 25(OH)D insufficiency in SSc that correlates with hip BMD (femoral neck and total femur) [39]. A significant correlation between vitamin D levels and BMD in the lumbar spine and femoral neck was described by ibn Yacoub et al. [37], with positive correlations of vitamin D levels and total femur BMD and femoral neck BMD, as reported by Sampaio-Barros et al. [32]. Furthermore, it seems that low levels of vitamin D represent a risk factor for fractures in scleroderma [38]. A detailed look into bone turnover biomarkers showed a significant negative correlation between serum C-telopeptide of type I collagen (CTX) and vitamin D in SSc [45]. In a comparative study with rheumatoid arthritis, Avouac et al. showed a higher prevalence of osteoporosis and fractures in patients with SSc and identified vitamin D concentrations, as well as age as risk factors, for such complications [38].

4.2.8. Quality of Life. The association of vitamin D and physical functioning in scleroderma is also of interest. Several papers have highlighted the relationship between hypovitaminosis D and physical functioning or quality of life, suggesting a role of decreased levels of vitamin D in worse quality of life. Sampaio-Barros et al. revealed a positive correlation for suboptimal vitamin D levels with quality of life questionnaires, including the SF-36-Vitality, SF-36-Social Function, SF-36-Emotional Role, and SF-36-Mental Health in SSc [32]. Moreover, a negative correlation between 25(OH)D and quality of life was demonstrated particularly in dcSSc, based on the HAQ-Reac and HAQ-Grip Strength. Calzolari et al. showed a significant correlation between vitamin D and physical performance score, assessed by the Medical Outcomes Study Short Form-36 (SF-36) questionnaire [45]. On the other hand, Vacca et al. found no association between vitamin D deficiency and the Health Assessment Questionnaire parameters [6].

4.2.9. Comorbidities/Complications of SSc. Other autoimmune diseases, such as primary biliary cirrhosis, secondary Sjögren syndrome, or thyroid diseases (including Hashimoto's thyroiditis and Graves' disease), are more frequent in SSc patients. Hypothyroidism may develop in up to 15% of patients with SSc, especially lcSSc, which is probably related to thyroid fibrosis [60]. Moreover, thyroid dysfunction may also have an important impact on clinical manifestations of SSc. Raynaud's phenomenon is more difficult to control in hypothyroid patients, and pulmonary hypertension can be severely influenced by hemodynamic changes in hypothyroidism [61].

Toki et al. identified 30 autoimmune thyroid disorder patients out of 210 SSc participants and reported hypothyroidism as more common [62]. Of interest, Giuggioli et al. described a strong association between hypovitaminosis D and autoimmune thyroiditis [27].

Periodontitis is one of the most common inflammatory diseases of adults and represents a chronic oral multibacterial infection [63]. Isola et al., in their clinical trial, found a negative association between periodontitis and vitamin D concentrations in SSc patients. Furthermore, the study emphasized lower vitamin D status in individuals with periodontitis and those with periodontitis and SSc, but not in healthy controls [11].

4.2.10. Serology. A specific antibody signature is known to occur in scleroderma patients, with high levels of circulating antinuclear antibodies (ANAs) associated with positivity for anti-centromere, anti-topoisomerase I (anti-SCL-70), and anti-RNA polymerase III antibodies. There is a close relationship between disease phenotype, clinical features, and antibody profile, with phenotypes supporting triple diagnostics, future organ involvement, and prognostic value [53, 64, 65].

The link between abnormal serum vitamin D and serologic specificities in SSc is still under debate. Several studies have reported that lower levels are significantly associated with positive antinuclear antibodies [36], anti-topoisomerase I antibodies [32], and anti-centromere antibodies [6]. In contrast, Gambichler et al. indicated no significant relationship between vitamin D and antinuclear autoantibodies [25, 41]. Moreover, Corrado et al. reported low vitamin D levels are independent of the presence of anti-topoisomerase I and anti-centromere autoantibodies in SSc patients [34].

Much attention has recently been given to CD34+ CD45- endothelial progenitor cells as a marker of early vascular activation and endothelial lesions in SSc, which could potentially be useful for disease activity estimation [66]. In a recent study, Lo Gullo et al. found an inverse correlation between vitamin D levels and CD34+ cell numbers in SSc patients [10].

Furthermore, it seems that vitamin D is able to increase IL-10 secretion by CD4+ T cells and enhances the number of IL-10-producing T regulatory T (Treg) cells, which are known to suppresses the immune response and mediate

immune tolerance [7]. Liberto et al. observed a significantly increased production of Treg cells and IL-10 following 1,25(OH) vitamin D treatment, suggesting a beneficial role of vitamin D supplementation in autoimmunity [17]. In a case-control study, Hax et al. demonstrated that IL-2 and IL-4 serum levels were reduced in SSc patients compared with controls; however, no significant correlation between cytokines and serum levels of vitamin D was demonstrated in such patients [12].

There are some limitations of this systematic literature review. Firstly, only PubMed was systematically search; other databases, such as Cochrane Library and Medline/EMBASE, were not included due to access restrictions. However, a high number of overlaps between these databases should have limited the number of potentially missed papers. Secondly, studies with different designs were included—prospective and retrospective, cross-sectional, case-control studies, and one meta-analysis—which may have resulted in bias in conclusions on the true role of vitamin D in the systemic sclerosis pathobiology.

5. Conclusions

In conclusion, hypovitaminosis D is common in systemic sclerosis patients, irrespective of the clinical and serological phenotype.

Because of the pleiotropic roles of vitamin D, including its immunomodulatory, cardioprotective, and antifibrotic properties, suboptimal vitamin D could interfere with all major pathobiological SSc pathways. Additionally, poor vitamin D status may be involved in clinical manifestations and SSc evolution, as shown in this literature review. However, details on the interference between low serum vitamin D levels and SSc pathogenesis remain controversial in some aspects, and future studies are needed to clarify this involvement.

If and when vitamin D levels should be monitored during the course of SSc, as well the significance and optimal dose of vitamin D supplementation, remain open questions.

Data Availability

The data supporting this review are from previously reported studies, which have been cited.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

All authors contributed equally to this work.

References

[1] M. Hughes, Y. Allanore, C. P. Denton, and M. Matucci-Cerinic, "Systemic sclerosis," *EMJ Rheumatology*, vol. 7, no. 1, pp. 100–109, 2020.

[2] M. Orlandi, G. Lepri, A. Damiani et al., "One year in review 2020: systemic sclerosis," *Clinical and Experimental Rheumatology*, vol. 38, no. 125, pp. 3–17, 2020.

[3] S. Kumar, J. Singh, S. Rattan, A. J. DiMarino, S. Cohen, and S. A. Jimenez, "Review article: pathogenesis and clinical manifestations of gastrointestinal involvement in systemic sclerosis," *Alimentary Pharmacology & Therapeutics*, vol. 45, no. 7, pp. 883–898, 2017.

[4] J. A. Reynolds and I. N. Bruce, "Vitamin D treatment for connective tissue diseases: hope beyond the hype?," *Rheumatology (Oxford)*, vol. 56, no. 2, pp. 178–186, 2017.

[5] S. W. Chang and H. C. Lee, "Vitamin D and health - the missing vitamin in humans," *Pediatrics and Neonatology*, vol. 60, no. 3, pp. 237–244, 2019.

[6] A. Vacca, C. Cormier, M. Piras, A. Mathieu, A. Kahan, and Y. Allanore, "Vitamin D deficiency and insufficiency in 2 independent cohorts of patients with systemic sclerosis," *The Journal of Rheumatology*, vol. 36, no. 9, pp. 1924–1929, 2009.

[7] M. Umar, K. S. Sastry, and A. I. Chouchane, "Role of vitamin D beyond the skeletal function: a review of the molecular and clinical studies," *International Journal of Molecular Sciences*, vol. 19, no. 6, p. 1618, 2018.

[8] M. F. Holick, N. C. Binkley, H. A. Bischoff-Ferrari et al., "Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 7, pp. 1911–1930, 2011.

[9] R. Nair and M. Arun, "Vitamin D: the "sunshine" vitamin," *Journal of Pharmacology & Pharmacotherapeutics*, vol. 3, no. 2, pp. 118–126, 2012.

[10] A. Lo Gullo, G. Mandraffino, J. Rodríguez-Carrio et al., "Endocan and circulating progenitor cells in women with systemic sclerosis: association with inflammation and pulmonary hypertension," *Biomedicine*, vol. 9, no. 5, p. 533, 2021.

[11] G. Isola, G. Palazzo, A. Polizzi, P. Murabito, C. Giuffrida, and A. Lo Gullo, "Association of systemic sclerosis and periodontitis with vitamin D levels," *Nutrients*, vol. 13, no. 2, p. 705, 2021.

[12] V. Hax, A. A. Gasparin, L. Schneider et al., "Vitamin D and cytokine profiles in patients with systemic sclerosis," *Journal of Clinical Rheumatology*, vol. 26, no. 7, pp. 289–294, 2020.

[13] S. Paolino, G. Pacini, C. Schenone et al., "Nutritional status and bone microarchitecture in a cohort of systemic sclerosis patients," *Nutrients*, vol. 12, no. 6, p. 1632, 2020.

[14] J. J. González-Martín, M. Novella-Navarro, and E. Calvo-Aranda, "Endothelial dysfunction and subclinical atheromatosis in patients with systemic sclerosis," *Clinical and Experimental Rheumatology*, vol. 125, no. 3, pp. 48–52, 2020.

[15] Á. Horváth, E. Végh, A. Pusztai et al., "Complex assessment of bone mineral density, fracture risk, vitamin D status, and bone metabolism in Hungarian systemic sclerosis patients," *Arthritis Research & Therapy*, vol. 21, no. 1, p. 274, 2019.

[16] C. Caimmi, E. Bertoldo, A. Pozza et al., "Vitamin D serum levels and the risk of digital ulcers in systemic sclerosis: a longitudinal study," *International Journal of Rheumatic Diseases*, vol. 22, no. 6, pp. 1041–1045, 2019.

[17] D. Di Liberto, C. Scazzone, G. La Rocca et al., "Vitamin D increases the production of IL-10 by regulatory T cells in patients with systemic sclerosis," *Clinical and Experimental Rheumatology*, vol. 119, no. 4, pp. 76–81, 2019.

[18] C. Corallo, A. Fioravanti, and S. Tenti, "Sarcopenia in systemic sclerosis: the impact of nutritional, clinical, and laboratory

features," *Rheumatology International*, vol. 39, no. 10, pp. 1767–1775, 2019.

[19] S. Gupta, V. K. Mahajan, R. S. Yadav et al., "Evaluation of serum vitamin D levels in patients with systemic sclerosis and healthy controls: results of a pilot study," *Indian Dermatology Online Journal*, vol. 9, no. 4, pp. 250–255, 2018.

[20] A. Taylan, M. Birlik, G. Kenar et al., "Osteoprotegrin interacts with biomarkers and cytokines that have roles in osteoporosis, skin fibrosis, and vasculopathy in systemic sclerosis: a potential multifaceted relationship between OPG/RANKL/TRAIL and Wnt inhibitors," *Modern Rheumatology*, vol. 29, no. 4, pp. 619–624, 2019.

[21] J. P. Kotyla, A. Kruszec-Zytniewska, A. J. Owczarek, M. Olszanecka-Glinianowicz, and J. Chudek, "Fibroblast growth factor 23 to alpha-klotho index correlates with systemic sclerosis activity: a proposal for novel disease activity marker," *Journal of Clinical Medicine*, vol. 7, no. 12, p. 558, 2018.

[22] A. C. Trombetta, V. Smith, E. Gotelli et al., "Vitamin D deficiency and clinical correlations in systemic sclerosis patients: a retrospective analysis for possible future developments," *PLoS One*, vol. 12, no. 6, p. e0179062, 2017.

[23] R. Ahmadi, M. Hajialilo, A. Ghorbanihaghjo et al., "FGF-23, klotho and vitamin D levels in scleroderma," *Iranian Journal of Public Health*, vol. 46, no. 4, pp. 530–536, 2017.

[24] L. An, M. H. Sun, F. Chen, and J. R. Li, "Vitamin D levels in systemic sclerosis patients: a meta-analysis," *Drug Design, Development and Therapy*, vol. Volume 11, pp. 3119–3125, 2017.

[25] M. Hajialilo, P. Noorabadi, S. Tahsini Tekantapeh, and A. Malek Mahdavi, "Endothelin-1, α-klotho, 25(OH) Vit D levels and severity of disease in scleroderma patients," *Rheumatology International*, vol. 37, no. 10, pp. 1651–1657, 2017.

[26] L. Zhang, Y. Duan, T. P. Zhang et al., "Association between the serum level of vitamin D and systemic sclerosis in a Chinese population: a case control study," *International Journal of Rheumatic Diseases*, vol. 20, no. 8, pp. 1002–1008, 2017.

[27] D. Giuggioli, M. Colaci, G. Cassone et al., "Serum 25-OH vitamin D levels in systemic sclerosis: analysis of 140 patients and review of the literature," *Clinical Rheumatology*, vol. 36, no. 3, pp. 583–590, 2017.

[28] E. K. Park, J. H. Park, S. M. Kweon, G. T. Kim, and S. G. Lee, "Vitamin D deficiency is associated with digital ulcer but not with atherosclerosis or arterial stiffness in patients with systemic sclerosis: a pilot study," *Clinical Rheumatology*, vol. 36, no. 6, pp. 1325–1333, 2017.

[29] M. P. Cruz-Domínguez, G. García-Collinot, M. A. Saavedra et al., "Clinical, biochemical, and radiological characterization of the calcinosis in a cohort of Mexican patients with systemic sclerosis," *Clinical Rheumatology*, vol. 36, no. 1, pp. 111–117, 2017.

[30] L. Groseanu, V. Bojinca, T. Gudu et al., "Low vitamin D status in systemic sclerosis and the impact on disease phenotype," *Eur J Rheumatol.*, vol. 3, no. 2, pp. 50–55, 2016.

[31] M. Atteritano, D. Santoro, and G. Corallo, "Skin involvement and pulmonary hypertension are associated with vitamin D insufficiency in scleroderma," *International Journal of Molecular Sciences*, vol. 17, no. 12, p. 2103, 2016.

[32] M. M. Sampaio-Barros, L. Takayama, P. D. Sampaio-Barros, E. Bonfá, and R. M. Pereira, "Baixos niveis sericos de vitamina D na esclerose sistemica difusa: correlaçao com pior qualidade de vida e alteraçoes capilaroscopicas graves," *Rev Bras Reumatol Engl Ed.*, vol. 56, no. 4, pp. 337–344, 2016.

[33] N. N. Carmel, P. Rotman-Pikielny, A. Lavrov, and Y. Levy, "Vitamin D antibodies in systemic sclerosis patients: findings and clinical correlations," *The Israel Medical Association Journal*, vol. 17, no. 2, pp. 80–84, 2015.

[34] A. Corrado, R. Colia, A. Mele et al., "Correction: relationship between body mass composition, bone mineral density, skin fibrosis and 25(OH) vitamin D serum levels in systemic sclerosis," *PLoS One*, vol. 10, no. 11, 2015.

[35] M. Atteritano, S. Sorbara, G. Bagnato et al., "Bone mineral density, bone turnover markers and fractures in patients with systemic sclerosis: a case control study," *PLoS One*, vol. 8, no. 6, p. e66991, 2013.

[36] R. Rios-Fernández, J. L. Callejas-Rubio, C. Fernández-Roldán et al., "Bone mass and vitamin D in patients with systemic sclerosis from two Spanish regions," *Clinical and Experimental Rheumatology*, vol. 30, no. 6, pp. 905–911, 2012.

[37] Y. Ibn Yacoub, B. Amine, A. Laatiris, F. Wafki, F. Znat, and N. Hajjaj-Hassouni, "Bone density in Moroccan women with systemic scleroderma and its relationships with disease-related parameters and vitamin D status," *Rheumatology International*, vol. 32, no. 10, pp. 3143–3148, 2012.

[38] J. Avouac, E. Koumakis, E. Toth et al., "Increased risk of osteoporosis and fracture in women with systemic sclerosis: a comparative study with rheumatoid arthritis," *Arthritis Care and Research*, vol. 64, no. 12, pp. 1871–1878, 2012.

[39] S. K. Shinjo, E. Bonfá, V. de Falco Caparbo, and R. M. Pereira, "Low bone mass in juvenile onset sclerosis systemic: the possible role for 25-hydroxyvitamin D insufficiency," *Rheumatology International*, vol. 31, no. 8, pp. 1075–1080, 2011.

[40] Y. Arnson, H. Amital, N. Agmon-Levin et al., "Serum 25-OH vitamin D concentrations are linked with various clinical aspects in patients with systemic sclerosis: a retrospective cohort study and review of the literature," *Autoimmunity Reviews*, vol. 10, no. 8, pp. 490–494, 2011.

[41] T. Gambichler, I. Chrobok, S. Höxtermann, and A. Kreuter, "Significantly decreased serum 25-hydroxyvitamin D levels in a large german systemic sclerosis cohort," *The Journal of Rheumatology*, vol. 38, no. 11, pp. 2492–2494, 2011.

[42] B. Seriolo, L. Molfetta, and M. Cutolo, "Seasonal variations in serum levels of 25-hydroxyvitamin D in patients with systemic sclerosis," *Clinical Rheumatology*, vol. 30, no. 3, pp. 445-446, 2011.

[43] P. Caramaschi, A. Dalla Gassa, O. Ruzzenente et al., "Very low levels of vitamin D in systemic sclerosis patients," *Clinical Rheumatology*, vol. 29, no. 12, pp. 1419–1425, 2010.

[44] R. Rios Fernández, C. Fernández Roldán, J. L. Callejas Rubio, and N. Ortego Centeno, "Vitamin D deficiency in a cohort of patients with systemic scleroderma from the south of Spain," *The Journal of Rheumatology*, vol. 37, no. 6, p. 1355, 2010.

[45] G. Calzolari, V. Data, R. Carignola, and A. Angeli, "Hypovitaminosis D in systemic sclerosis," *The Journal of Rheumatology*, vol. 36, no. 12, pp. 2844.2–282844, 2009.

[46] Y. Braun-Moscovici, D. E. Furst, D. Markovits et al., "Vitamin D, parathyroid hormone, and acroosteolysis in systemic sclerosis," *The Journal of Rheumatology*, vol. 35, no. 11, pp. 2201–2205, 2008.

[47] M. M. Hulshof, J. N. B. Bavinck, W. Bergman et al., "Double-blind, placebo-controlled study of oral calcitriol for the treatment of localized and systemic scleroderma," *Journal of the*

American Academy of Dermatology, vol. 43, no. 6, pp. 1017–1023, 2000.

[48] P. Humbert, J. L. Dupond, P. Agache, C. Drobacheff, B. de Wazieres, and F. Aubin, "Treatment of scleroderma with oral 1,25-dihydroxyvitamin D3: evaluation of skin involvement using non-invasive techniques. Results of an open prospective trial," *Acta Dermato-Venereologica*, vol. 73, no. 6, 1993.

[49] G. Kumánovics, M. Péntek, S. Bae et al., "Assessment of skin involvement in systemic sclerosis," *Rheumatology*, vol. 56, supplement 5, pp. v53–v66, 2017.

[50] A. L. Herrick and F. M. Wigley, "Raynaud's phenomenon," *Best Practice & Research: Clinical Rheumatology*, vol. 34, no. 1, article 101474, 2020.

[51] V. Devgire and M. Hughes, "Raynaud's phenomenon," *British Journal of Hospital Medicine*, vol. 80, no. 11, pp. 658–664, 2019.

[52] M. Hughes and A. L. Herrick, "Systemic sclerosis," *British Journal of Hospital Medicine*, vol. 80, no. 9, pp. 530–536, 2019.

[53] A. Sierra-Sepúlveda, A. Esquinca-González, S. A. Benavides-Suárez et al., "Systemic sclerosis pathogenesis and emerging therapies, beyond the fibroblast," *BioMed Research International*, vol. 2019, Article ID 4569826, 15 pages, 2019.

[54] A. Emmanuel, "Current management of the gastrointestinal complications of systemic sclerosis," *Journal of Scleroderma and Related Disorders*, vol. 1, no. 3, pp. 247–256, 2016.

[55] A. B. Shreiner, C. Murray, and C. Denton, "Gastrointestinal manifestations of systemic sclerosis," *Journal of Scleroderma and Related Disorders*, vol. 1, no. 3, pp. 247–256, 2016.

[56] C. P. Denton and D. Khanna, "Systemic sclerosis," *Lancet*, vol. 390, no. 10103, pp. 1685–1699, 2017.

[57] M. Chrabaszcz, J. Małyszko, M. Sikora et al., "Renal involvement in systemic sclerosis: an update," *Kidney and Blood Pressure Research*, vol. 45, no. 4, pp. 532–548, 2020.

[58] T. G. Woodworth, Y. A. Suliman, W. Li, D. E. Furst, and P. Clements, "Scleroderma renal crisis and renal involvement in systemic sclerosis," *Nature Reviews Nephrology*, vol. 12, no. 11, pp. 678–691, 2016.

[59] A. J. Cruz-Jentoft, J. P. Baeyens, J. M. Bauer et al., "Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People," *Age and Ageing*, vol. 39, no. 4, pp. 412–423, 2010.

[60] R. Adigun, A. Goyal, P. Bansal, and A. Hariz, *Systemic sclerosis*, StatPearls, 2021.

[61] A. Antonelli, C. Ferri, P. Fallahi et al., "Clinical and subclinical autoimmune thyroid disorders in systemic sclerosis," *European Journal of Endocrinology*, vol. 156, no. 4, pp. 431–437, 2007.

[62] S. Toki, S. Motegi, K. Yamada, A. Uchiyama, and O. Ishikawa, "Demographic and clinical features of autoimmune thyroid disorder in Japanese patients with systemic sclerosis," *The Journal of Dermatology*, vol. 41, no. 12, pp. 1053–1057, 2014.

[63] S. Hashioka, K. Inoue, M. Hayashida, R. Wake, A. Oh-Nishi, and T. Miyaoka, "Implications of systemic inflammation and periodontitis for major depression," *Frontiers in Neuroscience*, vol. 12, 2018.

[64] M. Kuwana, "Circulating anti-nuclear antibodies in systemic sclerosis: utility in diagnosis and disease subsetting," *Journal of Nippon Medical School*, vol. 84, no. 2, pp. 56–63, 2017.

[65] A. Stochmal, J. Czuwara, M. Trojanowska, and L. Rudnicka, "Antinuclear antibodies in systemic sclerosis: an update," *Clinical Reviews in Allergy & Immunology*, vol. 58, no. 1, pp. 40–51, 2020.

[66] M. Orlandi, S. Barsotti, G. Lepri et al., "One year in review 2018: systemic sclerosis," *Clinical and Experimental Rheumatology*, vol. 113, no. 4, pp. 3–23, 2018.

Hindawi
Journal of Immunology Research
Volume 2021, Article ID 9947370, 10 pages
https://doi.org/10.1155/2021/9947370

Research Article

Vitamin D Regulates the Expression of Glucocorticoid Receptors in Blood of Severe Asthmatic Patients

Bassam Mahboub,[1,2] Saba Al Heialy,[3,4] Mahmood Yaseen Hachim,[3] Rakhee K. Ramakrishnan,[1] Ashraf Alzaabi,[5] Rania Medhat Seliem,[2] Laila Ibraheem Salameh,[1,2] Sameen Masooma Toor,[5] Fathelrahman Salem Shendi,[2] Ola Mohamed Al Ali,[2] Basil Khalid Safarini,[2] Wafa Taleb Erabia,[2] Rabih Halwani,[1,6] and Qutayba Hamid[1,4]

[1]*Sharjah Institute for Medical Research, College of Medicine, University of Sharjah, Sharjah, UAE*
[2]*Rashid Hospital, Dubai Health Authority, Dubai, UAE*
[3]*Department of Basic Medical Sciences, College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, UAE*
[4]*Meakins-Christie Laboratories, The Research Institute of McGill University Health Centre and the Department of Medicine, McGill University, Montreal, QC, Canada*
[5]*Zayed Military Hospital, Abu Dhabi, UAE*
[6]*Prince Abdullah Ben Khaled Celiac Disease Chair, Department of Pediatrics, Faculty of Medicine, King Saud University, Saudi Arabia*

Correspondence should be addressed to Qutayba Hamid; qalheialy@sharjah.ac.ae

Received 1 April 2021; Accepted 17 July 2021; Published 6 August 2021

Academic Editor: Vlad Padureanu

Purpose. Vitamin D (VitD) deficiency is a significant public health concern in many areas around the globe and has been associated with many immune-mediated diseases, including asthma. Severe asthma has been linked to a decreased glucocorticoid receptor (GR) ratio (GR-α/GR-β ratio), indicating steroid hyporesponsiveness. Using a combination of *in silico* and *in vivo* approaches, we aimed to explore the immunomodulatory effect of VitD on asthmatic patients diagnosed with hypovitaminosis D. *Methods*. *In silico* tools were used to identify the regulatory effect of VitD supplementation on GR genes. We measured the expression levels of GR-α and the inactive isoform, GR-β, in the blood of adult asthmatics diagnosed with hypovitaminosis D before and after VitD supplementation. Moreover, the blood levels of inflammatory cytokines associated with asthma severity were determined. *Results*. Using an *in silico* approach, we identified specific genes commonly targeted by VitD as well as corticosteroids, the mainstay of asthma therapy. NR3C1 gene encoding GR was found to be significantly upregulated on Th2 CD4 cells and NK cells. Interestingly, blood expression level of NR3C1 was lower in severe asthmatics compared to nonsevere asthmatics and healthy controls, while the blood level of VitD receptor (VDR) was higher. Upon VitD supplementation of severe asthmatic patients, there was a significant increase in the blood levels of GR-α with no change in GR-β mRNA expression. VitD supplementation also suppressed the blood levels of IL-17F and IL-4. *Conclusion*. VitD may enhance steroid responsiveness by upregulating the expression of steroid receptor GR-α.

1. Introduction

Vitamin D (VitD) deficiency is a public health concern affecting all age groups and up to 80% of the population in certain countries [1]. Hypovitaminosis D is prevalent in the Middle East and North Africa (MENA) [2], and VitD level is suboptimal in the residents of sunny United Arab Emirates (UAE) [3]. In addition to its well-established role in calcium

and bone metabolism, the potential roles of VitD in cancer development, cardiovascular, and chronic lung diseases are being increasingly appreciated [4]. The increased awareness of VitD deficiency and related health problems have made vitamin D supplementation a routine action plan, albeit without laboratory confirmation in some cases leading to a higher risk of exogenous hypervitaminosis D [5]. Black and Scragg were among the first to show a positive correlation between VitD status and pulmonary function [6]. VitD deficiency is associated with features of asthma, such as decreased lung function, increased airway remodeling, and airway hyperresponsiveness [7]. Several reports have suggested the association of VitD levels with the severity of asthma [8]. Different cross-sectional studies further indicated that low serum VitD in patients with mild-to-moderate asthma is associated with poor asthma control, more exacerbations, reduced lung function, and increased medication use [9–11]. Inverse relationships have also been reported between serum VitD levels and airway remodeling, IgE, and eosinophil numbers, as well as airway hyperresponsiveness. Interestingly, Gupta et al. demonstrated a role for active VitD in correcting IL-10 levels in a pediatric population of moderate and severe therapy-resistant asthma [12]. On the other hand, experimental studies demonstrated that vitamin D receptor- (VDR-) deficient mice fail to develop airway inflammation (decreased infiltration of lymphocytes and eosinophils) and experimental allergic asthma [13].

Steroid resistance, as documented in cases of severe asthma, is associated with increased expression of the glucocorticoid receptor- (GR-) β, which is an alternative splicing isoform and dominant-negative regulator of GR-α [14]. The abundance of GR-α mRNA is higher than GR-β mRNA in all normal tissues and cells. Normally, the default splicing pathway leads to GR-α, while the alternative splicing pathway that leads to GR-β is minimally activated [15]. The latter pathway, however, seems to be upregulated in severe asthmatic patients [16]. In chronic inflammatory diseases like asthma, upregulated blood levels of inflammatory cytokines can participate in glucocorticoid resistance and impair GR function by modulating its translocation, DNA binding, and GR phosphorylation status.

As data from observational studies suggested a better control of asthma following restoration of VitD levels, we hypothesized that correcting VitD levels in adult asthmatic patients could enhance steroid responsiveness by regulating the blood GR-α/GR-β ratio.

2. Materials and Methods

2.1. In Silico Identification of Top Genes Regulated by Vitamin D Supplementation. We used Comparative Toxicogenomics Database to identify the top genes influenced by vitamin D supplementation in experiments. One hundred and five genes with at least five documented interactions with VitD were identified. Moreover, to explore if the identified genes shared common pathways, Enriched Ontology Clustering for the identified genes was performed using Metascape (a web-based tool used for comprehensive gene list annotation and analysis resource) [17].

2.2. In Silico Determination of NR3C1, NR3C2, and VDR mRNA Expression Levels in Immune Cells. Using the publicly available "Database of Immune Cell Expression, Expression quantitative trait loci (eQTLs) and Epigenomics," the NR3C1, NR3C2, and VDR mRNA expression levels in different immune cells were explored.

2.3. In Silico Determination of the mRNA Expression Levels and Correlations of NR3C1, NR3C2, and VDR in the Blood of Asthmatic Patients Compared to Healthy Controls. We extracted the expression profile of blood from the publicly available U-BIOPRED dataset (GSE69683), which included a sizable number of participants and their detailed clinical information. From a total of 498 participants, data from nonsmokers, which included 87 healthy controls, 77 patients with moderate asthma, and 246 severe asthmatics, were collected while those from 88 smoking severe asthmatics were excluded. Gene expression profiling of blood from these groups was analyzed, and the gene expression of GRs (NR3C1 and NR3C2) and VDR was determined.

2.4. Patients. A double-blinded, randomized, placebo-controlled study of VitD supplementation on 54 asthmatics with VitD deficiency was performed. Moderate-to-severe asthmatics between 18 and 65 years of age with clinician-diagnosed asthma and 25-hydroxyvitamin D3 (25D3) levels less than 20 ng/mL at the screening visit were recruited at the Rashid Hospital, Dubai, UAE, and the Zayed Military Hospital, Abu Dhabi, UAE. The patients' hospital electronic records were retrieved, and subjects were excluded if they had any previous VitD supplementation, had any other respiratory disease or comorbid conditions, or were smokers. According to the American Academy of Family Physicians guidelines, participants received 50,000 IU of VitD orally or placebo weekly over 8 weeks. Subjects were blinded to treatment and allocated to receive a VitD dose or placebo by the randomization schedule.

2.5. Ethics Approval and Consent to Participate. The study protocol was approved by the Institutional Ethics Committee at both Rashid Hospital and Zayed Military Hospital (MRC-04/2012-06) in accordance with international standards (Declaration of Helsinki), and written informed consent was obtained from all participants. Participants had consented to the use of their collected data and samples.

2.6. mRNA Expression and Cytokine Expression in Blood. In line with the U-BIOPRED study, whole blood was collected in PAXgene tubes (Qiagen, Germany) for the isolation of cellular RNA. Blood specimens were assessed for mRNA expression of glucocorticoid receptors (GR-α and GR-β) using qRT-PCR. Bioplex multiplex immunoassay (Bio-Rad, CA, USA) was used to assess the protein expression of IL-17A, IL-17-F, IFN-γ, IL-4, IL-5, and TNF-α in serum obtained from patients before and after VitD treatment.

2.7. Statistical Analysis. Standard student's t-tests and one-way ANOVA were performed to test for statistical significance between data groups using GraphPad Prism 8 (GraphPad, San Diego, CA, USA). $p < 0.05$ was considered significant.

3. Results

3.1. The Top Vitamin D Regulated Pathways Are Involved in Steroid Response. To identify VitD targeted enriched pathways, we analyzed Comparative Toxicogenomics Database. Interestingly, the top enriched pathways regulated by VitD were involved in several metabolic and inflammatory processes, including response to steroids and lipopolysaccharide as well as interleukin-4, interleukin-13, and AGE-RAGE signaling (Figure S1). Many of these VitD targeted pathways were also targets of dexamethasone. These included pathways involved in inflammation, proliferation, and proapoptosis, besides others (Figure S1).

3.2. VDR, NR3C1, and NR3C2 Expression Levels in Different Types of Immune Cells. The level of expression of GR encoding gene NR3C1 (nuclear receptor subfamily 3 group C member 1), mineralosteroid receptor encoding gene (NR3C2) [18], and VDR was then determined in different immune cells. NR3C1 had a significantly higher level of expression compared to VDR and NR3C2 in different types of immune cells (Figure 1). Among the various immune cells, its expression was the highest on Th2 CD4 cells (215.9 TPM). Classical (94.5 TPM) and nonclassical monocytes (46.7 TPM) had the highest VDR expression among all inflammatory cells. Moreover, the expression of all three genes was higher in nonclassical monocytes compared to classical ones (2.0, 1.49, and 1.21 folds, respectively). Naïve CD8 T cells (74.1 TPM) and Th1/17 CD4 cells on the other hand (64.7 TPM) had the highest NR3C2 mRNA expression among all immune cells. Notably, activation of T cells seemed to induce the expression of VDR and NR3C1, but not NR3C2 genes. For example, activated CD4 T cells expressed higher VDR and NR3C1 (3.95 and 1.25, respectively) but lower NR3C2 (0.11-fold), compared to naïve CD4 T cells. Similarly, activated CD8 T cells expressed higher VDR and NR3C1 (10.6 and 1.47, respectively) but lower NR3C2 (0.12-fold), compared to naïve cells (Figure 1).

3.3. VDR and Steroid Receptor Genes Are Differentially Regulated in Asthma Relative to Severity. Our *in silico* analysis demonstrated activated T cells to upregulate VDR, while treatment with VitD suppressed the proliferation of these cells, as well as their ability to produce IFN-γ and IL-17 cytokines [19]. The level of expression of VDR in the blood of asthmatics and its association with the expression of steroid receptors relative to disease severity is largely unknown. To investigate that, we analyzed the expression profile of blood from the publicly available dataset (GSE69683) which included a sizable number of participants and their detailed clinical information. A total of 498 participants, of which 87 were healthy controls, 77 with moderate asthma, and 246 nonsmokers with severe asthma, were included from the U-BIOPRED study. The differential expression of NR3C1, NR3C2, and VDR genes in the blood of participants from all groups was then determined. Patients with moderate asthma had higher levels of NR3C1 mRNA expression compared to healthy controls, although not to a significant extent (log2 intensity 10.17 ± 0.29; $p = 0.07$) (Figure 2(a)). When compared to moderate asthmatics, the expression of this gene, however, was significantly downregulated in severe asthmatics (log2 intensity 10.14 ± 0.23 versus 10.26 ± 0.21; $p < 0.01$). NR3C2 mRNA expression was also significantly downregulated in severe asthmatics compared to moderate asthma (log2 intensity 5.397 ± 0.727 versus 5.62 ± 0.5011, $p < 0.01$) and to healthy controls (log2 intensity 5.575 ± 0.5894 versus 5.397 ± 0.727, $p = 0.03$) (Figure 2(b)). Moderate asthmatics showed higher NR3C2 mRNA expression than healthy controls, although the difference was not significant. On the other hand, VDR mRNA expression was significantly upregulated in severe asthmatics compared to healthy controls (log2 intensity 8.386 ± 0.46 versus 8.206 ± 0.44, $p < 0.01$), but not to moderate asthmatics (Figure 2(c)). Interestingly, NR3C1 mRNA expression correlated positively (Spearman $r = 0.11$, 95%confidence interval = 0.02007 to 0.1988, and $p = 0.01$) (Figure 2(d)), while NR3C2 expression correlated negatively (Spearman $r = -0.55$, 95%confidence interval = −0.5724 to -0.438, and $p < 0.0001$) with the levels of VDR expression in blood (Figure 2(e)).

3.4. Supplementation of Vitamin D Selectively Favours the Upregulation of GR-α Receptor. To validate the *in silico* findings, a double-blinded, randomized, placebo-controlled study was conducted to test the effect of VitD supplementation on 54 vitamin D-deficient asthmatics. Moderate-to-severe asthmatics between 18 and 65 years of age who were diagnosed with asthma and with VitD (25D3) levels less than 20 ng/mL at the screening visit were recruited at two hospitals in UAE (Rashid Hospital, Dubai, UAE, and Zayed Military Hospital, Abu Dhabi, UAE). The patients were divided into two groups, an experimental group and a placebo group. The baseline serum levels of VitD in the experimental and placebo groups were comparable (11.74 ± 0.77 ng/mL and 12.96 ± 1.29 ng/mL, respectively). The experimental group received 50,000 IU of VitD via oral supplementation weekly for eight weeks [20]. Following treatment, a 2.84 ± 0.01-fold increase in blood VitD levels (33.34 ± 2.04 ng/mL) was observed (Figure 3). The level of VitD in the placebo group did not change (14.45 ± 1.61 ng/mL) (Table 1 and Figure 3). This result indicated that the regimen used was effective in correcting VitD deficiency.

We next determined whether VitD supplementation affected the gene expression of steroid receptors, GR-α or GR-β. Correction of VitD levels led to a significant increase in the expression of GR-α when compared to the baseline levels. A significant increase was observed following treatment with VitD compared to pretreatment levels (1.89 ± 0.56 and 5.34 ± 1.40, respectively; $p < 0.05$) (Figure 4(a)). Moreover, there was a significant increase in GR-α expression in patients who received VitD compared to those who received the placebo control (5.34 ± 1.40 versus 1.64 ± 0.76-fold; $p < 0.05$). Interestingly, no change in GR-β expression was observed following treatment with VitD (1.49 ± 0.27 versus 1.30 ± 0.25) (Figure 4(b)). Relatively, the GR-α/GR-β ratio increased significantly following VitD supplementation compared to pretreatment levels (3.58 ± 0.83-fold; $p < 0.05$) or patients who received a placebo (Figure 4(c)).

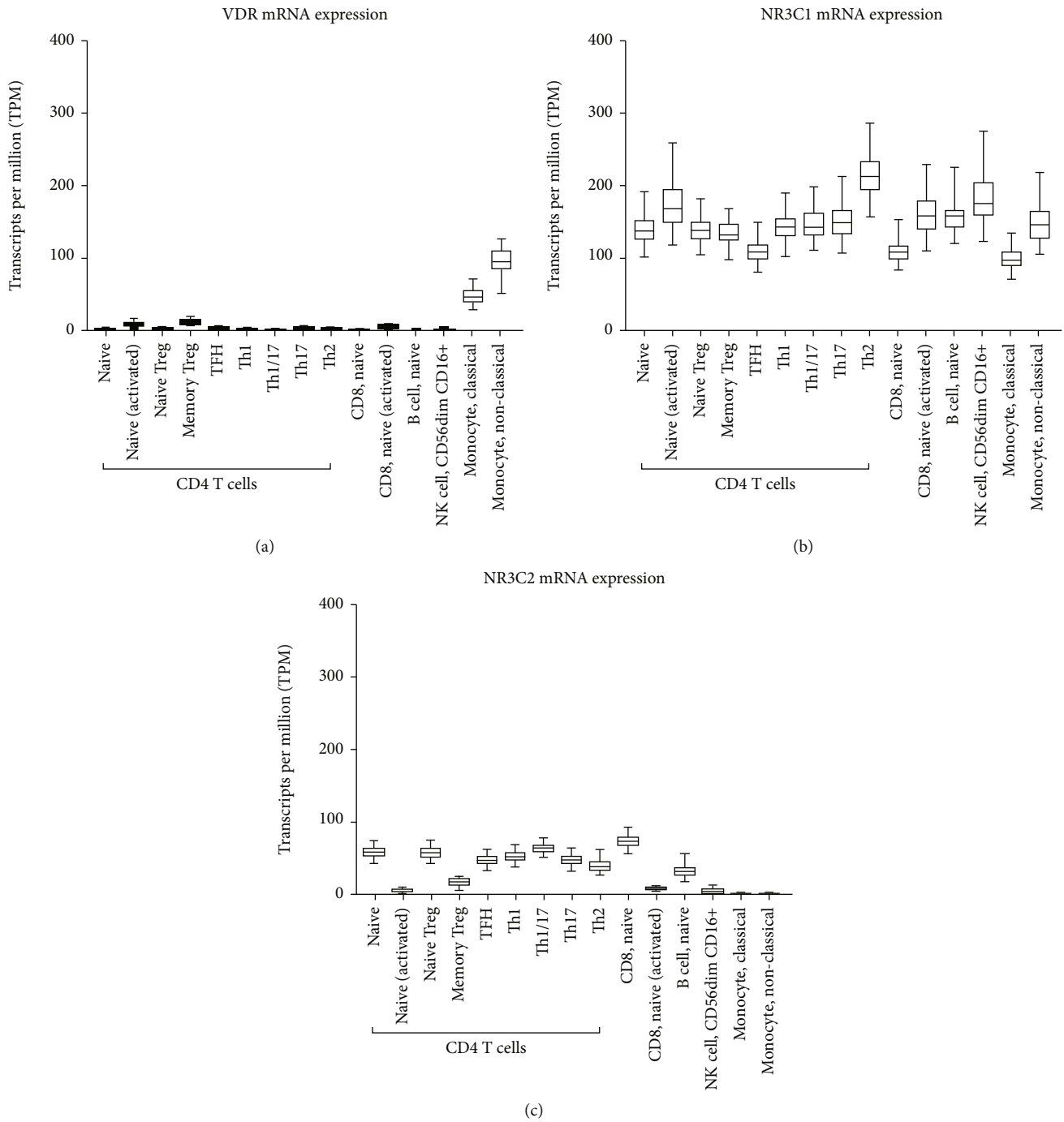


Figure 1: VDR, NR3C1, and NR3C2 mRNA expression levels in immune cells. Data was extracted using the publicly available "Database of Immune Cell Expression, Expression quantitative trait loci (eQTLs) and Epigenomics."

3.5. Vitamin D Supplementation Reduced the Blood Levels of IL-17 and IL-4 Cytokines in Asthmatic Patients. The effect of VitD supplementation on the blood levels of asthma-related proinflammatory cytokines was then determined (Figure 5) [21]. Blood levels of IL-17A, IL-17F, IFN-γ, IL-4, IL-5, and TNF-α were determined before and after VitD supplementation using ELISA assay (Figure 5). VitD preferentially suppressed the blood levels of IL-17F cytokine ($p = 0.04$) without affecting IL-17A, while there was no significant difference in the placebo group ($p = 0.17$). A decrease in blood IL-4 cytokine levels was also observed following treatment, however not to a significant level ($p = 0.07$). No significant effect of VitD supplementation was observed on the rest of the cytokines tested.

4. Discussion

This study highlights the role of VitD in glucocorticoid resistance, a significant feature of severe asthma. To our knowledge, this is the first study to determine such an effect of

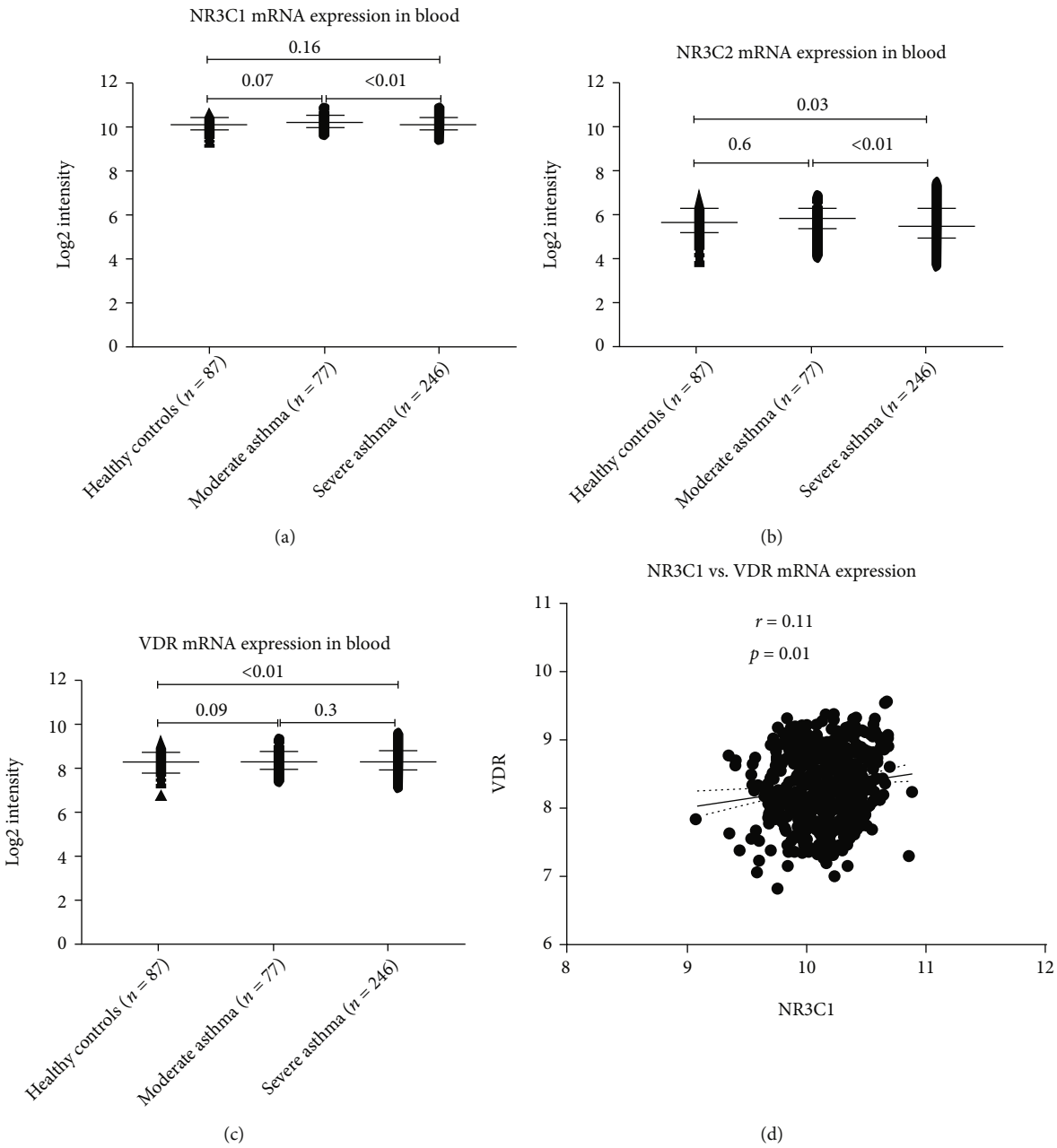
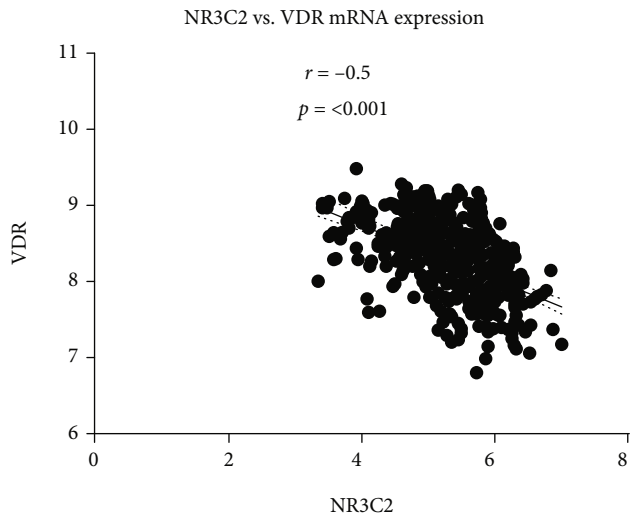


Figure 2: Continued.



(e)

Figure 2: NR3C1, NR3C2, and VDR mRNA expression levels in the blood of subjects with severe asthma, moderate asthma, and nonasthmatics. Data was collected from the U-BIOPRED study (GSE69683).

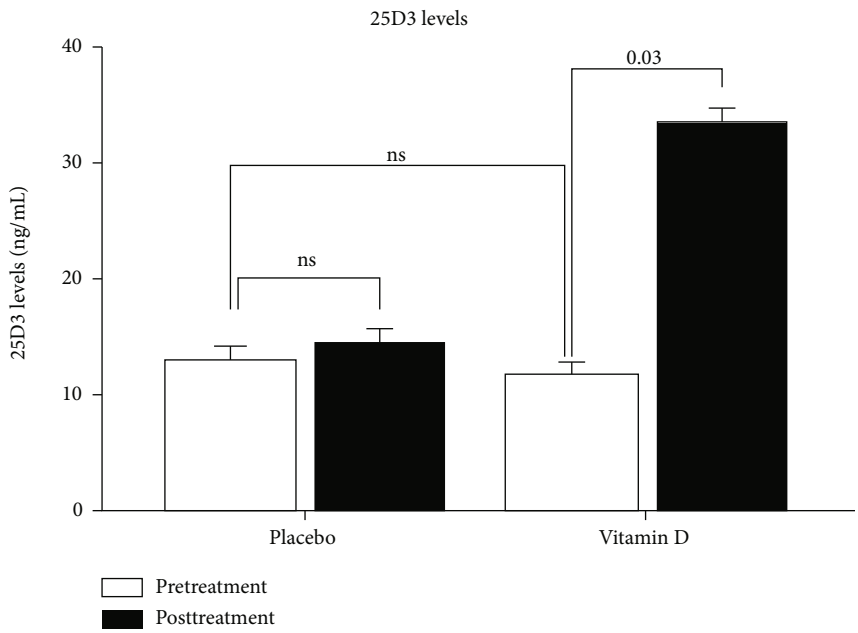


Figure 3: Pretreatment and posttreatment levels of serum vitamin D (25D3) in patients who received vitamin D or placebo for eight weeks. Asthmatic patients with vitamin D deficiency were divided into 2 groups. 23 patients received 50,000 IU of vitamin D orally over 8 weeks, while 22 patients received placebo. Vitamin D levels were measured in the blood of these patients before and after the treatment.

VitD supplementation on glucocorticoid receptor expression in adult asthmatics.

Epidemiological studies have provided early evidence of potential immunomodulatory effects of VitD [22, 23]. VitD binds to the VDR, a member of the superfamily of nuclear receptors expressed by all immune cells. This leads to alterations in the binding of VDR to vitamin D response elements (VDREs) and consequent changes in the transcription of VDRE-regulated genes [24]. By controlling gene expression through the VDR, 25D3, the active metabolite of vitamin D, could limit tissue inflammation [25]. Of interest, multiple lines of evidence have suggested a link between VitD and asthma, including genetic studies showing associations between VDR polymorphisms and asthma [26]. Several studies have reported polymorphisms of the VDR, which subsequently influenced asthma and allergy susceptibility [27, 28]. Animal studies in various disease models have also demonstrated the potent inhibition of proinflammatory chemokine synthesis upon vitamin D treatment [29–31]. Moreover, vitamin D may exert a protective modulatory effect on inflammation by reducing Th17 cytokines in peripheral blood mononuclear cells (PBMCs) and increasing the number of regulatory T cells [32]. The anti-inflammatory effects of vitamin D also include the reduction of TNF-α-induced

Table 1: Clinical characteristics of patients who received vitamin D or placebo for eight weeks.

Variable	Total	Placebo	Vitamin D
Total	54	20	34
Male	19 (35%)	4 (20%)	15 (44%)
Female	35 (64.8%)	16 (80%)	19 (55.8%)
Age (years)	38.64 ± 1.90	40.60 ± 2.97	37.45 ± 2.48
Body mass index	32.27 ± 0.92	32.32 ± 5.52	32.32 ± 1.28
Pretreatment 25D3 levels (ng/mL)		12.96 ± 1.29	11.74 ± 0.77
Posttreatment 25D3 levels (ng/mL)		14.45 ± 1.61	33.34 ± 2.04

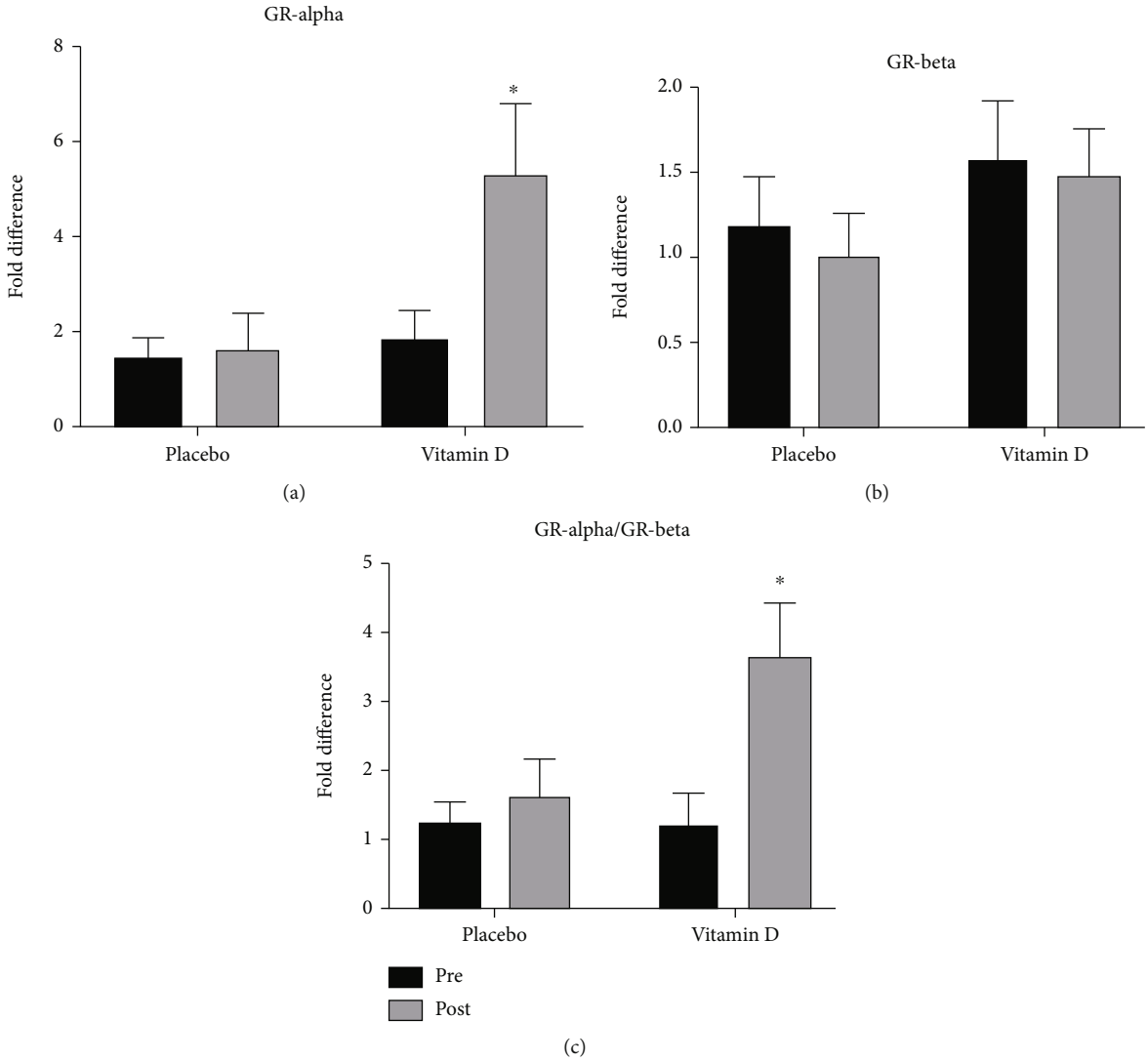


Figure 4: Vitamin D treatment increases the expression of GR-α with no effect on GR-β. Asthmatic subjects were treated with 50,000 IU of vitamin D orally or placebo for eight weeks. Blood specimens obtained prior to and following 8 weeks of treatment were analyzed for mRNA expression of (a) GR-α and (b) GR-β using RT-qPCR, and the ratio of (c) GR-α/GR-β was calculated. $^*p < 0.05$.

CCL5 and CXCL10 production by airway smooth muscle cells [33].

Using an *in silico* approach and by assessing the transcriptomic signatures of large asthmatic cohorts, we observed a significantly high level of expression of the gene-regulating GR-α and GR-β receptors, NR3C1, in most of the inflammatory cells tested. Th2 cells had the highest level of expression of NR3C1 compared to other inflammatory cells, which may explain the effective control by steroids of Th2 immune responses, a dominant feature in mild-to-moderate asthma. Interestingly, the expression of this steroid receptor coding gene in the blood of severe asthmatic patients was

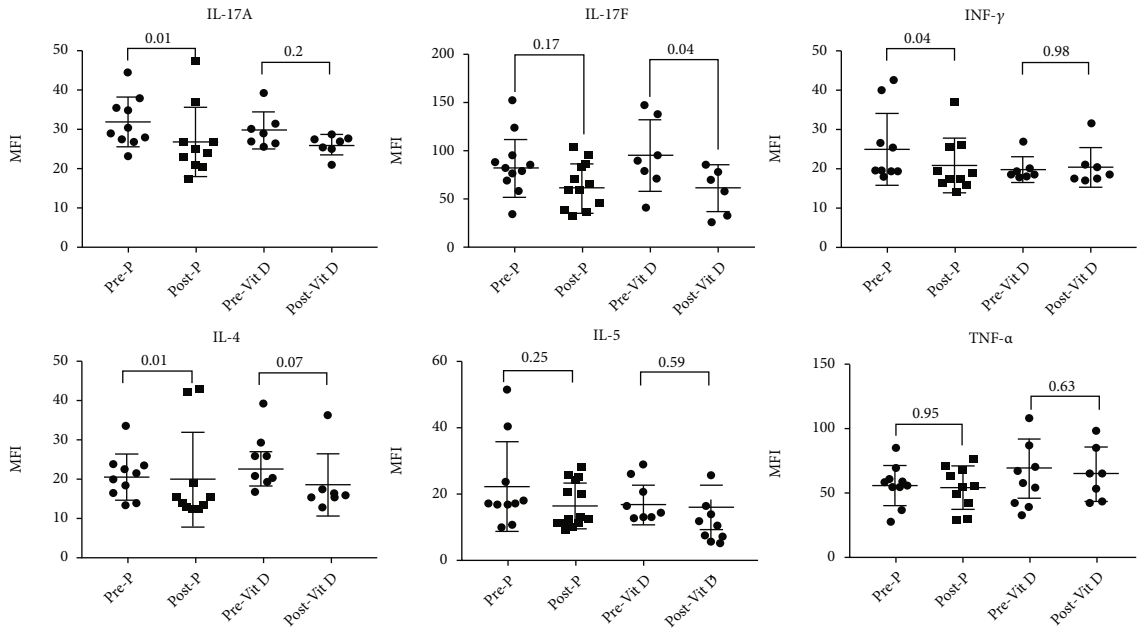


Figure 5: Cytokine profiles of pre- and postvitamin D treatment in the blood of severe asthmatics with vitamin D deficiency. Cytokine levels were measured in the blood of asthmatic patients before and after treatment with 50,000 IU of vitamin D or placebo using Bioplex assay, $n = 23$ for the vitamin D-treated group and $n = 22$ for the placebo group.

significantly lower than those of moderate asthmatic patients, which may explain the steroid hyporesponsiveness observed in severe conditions. VDR blood levels were higher in severe conditions, and a positive correlation was observed between VDR and NR3C1, but not NR3C2, which may suggest a possible regulatory association between them. Moreover, the enhanced expression of VDR in the blood of severe asthmatics may explain the observed *in vivo* response to VitD treatment.

To explore how vitamin D may regulate the response to steroids during asthma, we assessed the expression levels of glucocorticoid receptors, GR-α and GR-β, in VitD-deficient asthmatic patients before and after vitamin D supplementation. Interestingly, correcting VitD levels enhanced the GR-α/GR-β ratio by upregulating the levels of GR-α without affecting GR-β levels. VitD supplementation may selectively favour the upregulation of the default splicing pathway leading to an overall increase in GR-α expression, with no effect on the alternative splicing pathway, responsible for GR-β expression. This is in line with a previous study that reported a significant decrease in the expression of GR-β upon vitamin D treatment in subjects with low vitamin D level [34]. By increasing the expression of GR-α, VitD may hence rescue the steroid insensitivity encountered in severe asthmatics. Therefore, this study highlights the role of VitD in improving the response to steroids in severe asthmatics suffering from steroid resistance.

We also investigated the effect of VitD supplementation on the expression of proinflammatory cytokines involved in asthma pathogenesis, including Th2 cytokines (IL-4 and IL-5) and Th17 cytokines (IL-17A and IL-17F). Eight weeks of VitD treatment significantly suppressed the expression of IL-17F compared to pretreatment (Figure 5). Although the role of IL-17F in asthma pathogenesis is well documented [35], the mechanism regulating its expression is not very well understood. Recently, the suppression of IL-17F, but not of IL-17A, was found to protect against colitis by inducing Treg cells [36]. A similar mechanism could be involved in the observed anti-inflammatory effect of VitD. We have also observed a decrease in IL-4 blood levels following VitD supplementation, however not to a significant level. Being a key cytokine in asthma pathogenesis, the observed VitD-induced suppression of IL-4 levels may significantly contribute to the efficiency of VitD in controlling asthma pathogenesis. A previous report showed that VitD improved steroid-induced IL-10 production. VitD, however, did not affect IL-10, IL-13, or IL-17A production by PBMCs of moderate and steroid-resistant asthmatic patients [37], similar to what we observed in our cohort.

Some of the limitations of our study include the relatively small sample size. We were unable to follow up with a substantial proportion of our recruited patients after 8 weeks of VitD or placebo treatments as the patients failed to respond to our follow-up requests. This significantly impacted our sample size resulting in a pilot proof-of-concept study.

In conclusion, restoring VitD levels may improve the response of severe asthmatic patients to steroid treatment by enhancing the expression of GR-α. Our results highlight the importance of maintaining normal levels of vitamin D in controlling chronic airway inflammation.

Data Availability

All data generated or analyzed during this study are included in this manuscript.

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee at both Rashid Hospital and Zayed Military Hospital (MRC-04/2012-06). All methods were performed in accordance with the relevant guidelines and regulations.

Consent

Written informed consent was obtained from all participants. Participants had consented to the use of their collected data and samples.

Conflicts of Interest

The authors have no financial or nonfinancial competing interests to disclose.

Authors' Contributions

Conception or design of the work was contributed by QH, BM, and SA. Data collection was done by BM, AA, RMS, LIS, SMT, FSS, OMA, BKS, WTE, SH, and QH. Data analysis and interpretation was done by RKR, MYH, QH, BM, and SH. Drafting the article was carried out by all authors. Critical revision of the article was completed by QH, BM, SH, MYH, and RH. Final approval of the version to be published was made by all authors.

Acknowledgments

The authors would like to acknowledge the efforts and support provided by the administrative and clinical teams in DHA, Rashid Hospital, Zayed Military Hospital, Meakins-Christie Laboratories, the Research Institute of McGill University Health Centre, and the Sharjah Institute for Medical Research. We also acknowledge the support of Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences, Dubai, UAE, to BM and Prince Abdullah Ben Khalid Celiac Disease Research Chair, under the Vice Deanship of Research Chairs, King Saud University, Riyadh, Kingdom of Saudi Arabia, to RH.

Supplementary Materials

A supplementary figure file is included with this manuscript. Figure S1: functional enrichment, interactome analysis, gene annotation, drug signature, and membership search for the top 105 vitamin D target genes. *(Supplementary Materials)*

References

[1] C. Palacios and L. Gonzalez, "Is vitamin D deficiency a major global public health problem?," *The Journal of steroid biochemistry and molecular biology*, vol. 144, pp. 138–145, 2014.

[2] D. Bassil, M. Rahme, M. Hoteit, and G. E.-H. Fuleihan, "Hypovitaminosis D in the Middle East and North Africa: prevalence, risk factors and impact on outcomes," *Dermato-endocrinology*, vol. 5, no. 2, pp. 274–298, 2013.

[3] H. M. Al Attia and M. A. Ibrahim, "The high prevalence of vitamin D inadequacy and dress style of women in the sunny UAE," *Archives of Osteoporosis*, vol. 7, no. 1-2, pp. 307–310, 2012.

[4] H. Wang, W. Chen, D. Li et al., "Vitamin D and chronic diseases," *Aging and disease*, vol. 8, no. 3, pp. 346–353, 2017.

[5] E. Marcinowska-Suchowierska, M. Kupisz-Urbańska, J. Łukaszkiewicz, P. Płudowski, and G. Jones, "Vitamin D toxicity-a clinical perspective," *Frontiers in endocrinology*, vol. 9, pp. 550–550, 2018.

[6] P. N. Black and R. Scragg, "Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey," *Chest*, vol. 128, no. 6, pp. 3792–3798, 2005.

[7] R. E. Foong, N. C. Shaw, L. J. Berry, P. H. Hart, S. Gorman, and G. R. Zosky, "Vitamin D deficiency causes airway hyperresponsiveness, increases airway smooth muscle mass, and reduces TGF-β expression in the lungs of female BALB/c mice," *Physiological reports*, vol. 2, no. 3, article e00276, 2014.

[8] J. Cepeda, C. Fuentes, D. Zenteno, and R. Bustos, "Vitamin D and pediatrics respiratory diseases," *Revista chilena de pediatria*, vol. 90, no. 1, pp. 94–101, 2019.

[9] J. M. Brehm, J. C. Celedón, M. E. Soto-Quiros et al., "Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica," *American journal of respiratory and critical care medicine*, vol. 179, no. 9, pp. 765–771, 2009.

[10] J. M. Brehm, B. Schuemann, A. L. Fuhlbrigge et al., "Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study," *Journal of Allergy and Clinical Immunology*, vol. 126, no. 1, pp. 52–58.e5, 2010.

[11] I. Chinellato, M. Piazza, M. Sandri, D. Peroni, G. Piacentini, and A. L. Boner, "Vitamin D serum levels and markers of asthma control in Italian children," *The Journal of pediatrics*, vol. 158, no. 3, pp. 437–441, 2011.

[12] A. Gupta, A. Bush, C. Hawrylowicz, and S. Saglani, "Vitamin D and asthma in children," *Paediatric respiratory reviews*, vol. 13, no. 4, pp. 236–243, 2012.

[13] A. Wittke, V. Weaver, B. D. Mahon, A. August, and M. T. Cantorna, "Vitamin D receptor-deficient mice fail to develop experimental allergic asthma," *The journal of immunology*, vol. 173, no. 5, pp. 3432–3436, 2004.

[14] K. Scheschowitsch, J. Leite, and J. Assreuy, "New insights in glucocorticoid receptor signaling – more than just a ligand binding receptor," *Frontiers in endocrinology*, vol. 8, 2017.

[15] L. Pujols, J. Mullol, J. Roca-Ferrer et al., "Expression of glucocorticoid receptor alpha- and beta-isoforms in human cells and tissues," *American Journal of Physiology-Cell Physiology*, vol. 283, no. 4, pp. C1324–C1331, 2002.

[16] A. Vazquez-Tello, R. Halwani, Q. Hamid, and S. Al-Muhsen, "Glucocorticoid receptor-beta up-regulation and steroid resistance induction by IL-17 and IL-23 cytokine stimulation in peripheral mononuclear cells," *Journal of clinical immunology*, vol. 33, no. 2, pp. 466–478, 2013.

[17] Y. Zhou, B. Zhou, L. Pache et al., "Metascape provides a biologist-oriented resource for the analysis of systems-level datasets," *Nature communications*, vol. 10, article 9234, no. 1, p. 1523, 2019.

[18] H. Palma-Gudiel, A. Córdova-Palomera, J. C. Leza, and L. Fañanás, "Glucocorticoid receptor gene (*NR3C1*) methylation processes as mediators of early adversity in stress-related

disorders causality: a critical review," *Neuroscience & Biobehavioral Reviews*, vol. 55, pp. 520–535, 2015.

[19] M. T. Cantorna, L. Snyder, Y.-D. Lin, and L. Yang, "Vitamin D and 1,25(OH)$_2$D regulation of T cells," *Nutrients*, vol. 7, no. 4, pp. 3011–3021, 2015.

[20] K. A. Kennel, M. T. Drake, and D. L. Hurley, "Vitamin D deficiency in adults: when to test and how to treat," *mayo clinic proceedings*, vol. 85, no. 8, pp. 752–758, 2010.

[21] T. W. W. Pace, F. Hu, and A. H. Miller, "Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression," *Brain, behavior, and immunity*, vol. 21, no. 1, pp. 9–19, 2007.

[22] D. E. Roth, G. Soto, F. Arenas et al., "Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis," *Journal of Infectious Diseases*, vol. 190, no. 5, pp. 920–927, 2004.

[23] R. J. Wilkinson, M. Llewelyn, Z. Toossi et al., "Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study," *Lancet*, vol. 355, no. 9204, pp. 618–621, 2000.

[24] C. Carlberg and M. J. Campbell, "Vitamin D receptor signaling mechanisms: integrated actions of a well-defined transcription factor," *Steroids*, vol. 78, no. 2, pp. 127–136, 2013.

[25] M. Umar, K. S. Sastry, and A. I. Chouchane, "Role of vitamin D beyond the skeletal function: a review of the molecular and clinical studies," *International journal of molecular sciences*, vol. 19, no. 6, p. 1618, 2018.

[26] A. Papadopoulou, P. Kouis, N. Middleton et al., "Association of vitamin D receptor gene polymorphisms and vitamin D levels with asthma and atopy in Cypriot adolescents: a case-control study," *Multidisciplinary respiratory medicine*, vol. 10, no. 1, pp. 26–26, 2015.

[27] A. H. Poon, C. Laprise, M. Lemire et al., "Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy," *American journal of respiratory and critical care medicine*, vol. 170, no. 9, pp. 967–973, 2004.

[28] B. A. Raby, R. Lazarus, E. K. Silverman et al., "Association of vitamin D receptor gene polymorphisms with childhood and adult asthma," *American journal of respiratory and critical care medicine*, vol. 170, no. 10, pp. 1057–1065, 2004.

[29] N. Giarratana, G. Penna, S. Amuchastegui, R. Mariani, K. C. Daniel, and L. Adorini, "A vitamin D analog down-regulates proinflammatory chemokine production by pancreatic islets inhibiting T cell recruitment and type 1 diabetes development," *The Journal of Immunology*, vol. 173, no. 4, pp. 2280–2287, 2004.

[30] C. A. Gysemans, A. K. Cardozo, H. Callewaert et al., "1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice," *Endocrinology*, vol. 146, no. 4, pp. 1956–1964, 2005.

[31] L. B. Pedersen, F. E. Nashold, K. M. Spach, and C. E. Hayes, "1,25-Dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking," *Journal of neuroscience research*, vol. 85, no. 11, pp. 2480–2490, 2007.

[32] E. S. Chambers and C. M. Hawrylowicz, "The impact of vitamin D on regulatory T cells," *Current allergy and asthma reports*, vol. 11, no. 1, pp. 29–36, 2011.

[33] A. Banerjee, G. Damera, R. Bhandare et al., "Vitamin D and glucocorticoids differentially modulate chemokine expression in human airway smooth muscle cells," *British journal of pharmacology*, vol. 155, no. 1, pp. 84–92, 2008.

[34] S. N. Guner, R. Sancak, S. Gulten, A. Özen, M. Kilic, and A. Bedir, "Glucocorticoid receptor-β is downregulated by vitamin D treatment in children with low vitamin D levels but not in allergic asthma," *Pediatric Allergy, Immunology, and Pulmonology*, vol. 28, no. 3, pp. 144–151, 2015.

[35] S. H. Chang and C. Dong, "IL-17F: regulation, signaling and function in inflammation," *Cytokine*, vol. 46, no. 1, pp. 7–11, 2009.

[36] C. Tang, S. Kakuta, K. Shimizu et al., "Suppression of IL-17F, but not of IL-17A, provides protection against colitis by inducing T_{reg} cells through modification of the intestinal microbiota," *Nature Immunology*, vol. 19, no. 7, pp. 755–765, 2018.

[37] A. Gupta, S. Dimeloe, D. F. Richards et al., "Defective IL-10 expression and in vitro steroid-induced IL-17A in paediatric severe therapy-resistant asthma," *Thorax*, vol. 69, no. 6, pp. 508–515, 2014.