Review

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Vitamin D association with systemic sclerosis and its clinical features: A systematic review, meta-analysis, and meta-regression

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Abstract

Objectives: The aim of this review was to summarize existing data on the contribution of Vitamin D level and/or deficiency/insufficiency to systemic sclerosis susceptibility and its clinical features.

Methods: An electronic literature search for eligible studies among all papers published prior to 30 June 2024 was conducted through PubMed, EMBASE, Web of science, and Scopus databases. Meta-analyses estimating pooled raw mean differences, odds ratios, and Pearson *r* together with subgroup analyses and meta-regressions were performed for the association of Vitamin D with susceptibility to systemic sclerosis and disease presentation.

Results: Combined analysis revealed a significant decrease in Vitamin D level in systemic sclerosis patients comparatively to healthy controls, with raw mean differences 95% CI = −11.68 [−15.43 to −7.92] ng/mL, $p < 1$ E-10. Likewise, Vitamin D insufficiency (Vitamin D < 30 ng/mL) and deficiency (< 10 ng/mL) were significantly associated with systemic sclerosis; odds ratios 95% CI = 3.58 [2.59–4.95], $p < 1$ E-10 and odds ratios 95% CI = 7.67 [3.97–14.83], $p < 1$ E-10, respectively. Moreover, decreased Vitamin D level was significantly associated with interstitial lung disease occurrence (raw mean differences 95% CI = −3.61 [−6.93 to −0.3], $p = 0.003$), while Vitamin D deficiency was associated with increased systolic pulmonary arterial pressure, raw mean differences (95% CI = 4.17 [1.44–6.89], $p = 0.003$). Besides, Vitamin D level was negatively correlated with the modified Rodnan skin score, *r* (95% CI = −0.26 [−0.44 to −0.08], $p = 0.004$). Conversely, Vitamin D level was significantly increased in systemic sclerosis patients with cutaneous calcinosis, raw mean differences (95% CI = 4.18 [1.07–7.28], $p = 0.008$).

Conclusion: This meta-analysis showed that decreased Vitamin D level was associated with susceptibility to systemic sclerosis, interstitial lung disease occurrence, increased systolic pulmonary arterial pressure, and higher modified Rodnan skin score. Conversely, calcinosis was found to be associated with increased Vitamin D level.

Registration: This review has been registered on PROSPERO: CRD42024565045, available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024565045

Keywords

Systemic sclerosis, Vitamin D, systematic review, meta-analysis, meta-regression

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Introduction

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease characterized by extensive micro and macrovascular alterations, and widespread progressive fibrosis of the skin and various internal organs such as the lungs, digestive tract, and heart.[1–4] Two distinct clinical subsets are defined based on the extent of skin fibrosis:

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diffuse SSc (dSSc) and limited SSc (lSSc).[4] Several autoantibodies can be detected in SSc patients such as anti-Scl70 antibody (Scl70 Ab), anti-centromere Ab (ACA), anti-RNA polymerase III Ab, anti-Th/T0 Ab, anti-PM-Scl Ab, and anti-NOR90 Ab.[4] While Scl70 Ab is mainly detected in patients with dSSc, ACA is rather observed in lSSc cases.[4]

Like other systemic autoimmune diseases, SSc results from the combination of predisposing genetic factors, environmental factors, and hormonal factors since the disease mainly affects women.[4] The genetic background of SSc is complex, including both HLA and non-HLA genes.[5] Besides, there are numerous environmental triggers that have been identified in SSc, including occupational exposures, chemical materials, silicone implants, microorganisms, medications, and smoking habit.[6] Hence, it has been suggested that the concomitant association of the aforementioned factors may cause a loss of tolerance toward self-antigens with persistent immune system activation without efficient downregulation by regulatory T and B cells and immune checkpoints.[4] In this regard, Vitamin D (Vit D) receptors (VDRs), which are present in several immune cells, including antigen-presenting cells, natural killer cells, and activated T and B cells, might exert immunomodulatory actions during autoimmunity.[7–9]

Vit D is a steroid hormone precursor obtained from the skin following sunlight exposure, and in a small part from food intake.[7–9] Its active form, 1,25-dihydroxycholecalciferol (1,25(OH)2D3) or calcitriol, plays a critical role in calcium and phosphate balance.[8,9] In addition to its classical role in maintaining calcium homeostasis and bone health, Vit D exhibits pleiotropic effects since VDRs are ubiquitously expressed.[8,9] Indeed, Vit D was found to be implicated in countless physiological processes such as cell differentiation, proliferation, and apoptosis, angiogenesis, and innate and adaptive immune responses.[7–9] Regarding the modulation of immune responses, Vit D inhibits T cell proliferation, reduces proinflammatory cytokines production, and promotes the shift of T-helper cells from Th1 and Th17 to Th2 phenotype.[7–9] Hence, taking into account the immunomodulatory properties of Vit D, several studies have studied the association of Vit D with SSc and its clinical presentation over the past three decades. However, results were mostly inconsistent and observed heterogeneity needs to be extensively examined.

The aim of this review was to summarize existing data on the contribution of Vit D level and/or deficiency/insufficiency to SSc susceptibility and its clinical features, and to investigate the between-studies heterogeneity by subgroup analyses and meta-regressions.

Materials and methods

Search strategy

This study was performed according to the PRISMA guidelines for systematic reviews and meta-analyses.[10] An electronic literature search for eligible studies among all papers published prior to 30 June 2024 was conducted through PubMed, EMBASE, Web of science, and Scopus databases. The following search string was used: (((Systemic Sclerosis) OR (SSc) OR (Scleroderma)) AND ((Vitamin D) OR (Vitamin-D) OR (Vit D) OR (25-OH D) OR (25OHD) OR (25-hydroxyvitamin D3) OR (25(OH)D3) OR (25 OH Vitamin D) OR (25(OH) Vitamin D) OR (25(OH) Vit D) OR (25-OH-vitD))). The literature search was carried out without any language restriction. A detailed search strategy is available within Supplementary File 1.

Selection criteria

All studies were independently assessed and evaluated by two reviewers (T.D. and I.S.) for the inclusion and exclusion. The following selection criteria were adopted.

Inclusion criteria:

- Studies of case–control (retrospective) or cohort (prospective) design;
- Studies assessing the association of Vit D level and/or Vit D deficiency/insufficiency with SSc occurrence and/or its clinical features.

Exclusion criteria:

- Studies carried out in patients with morphea (localized scleroderma);
- Studies performed in patients followed for overlapping SSc with other connectivities;
- Case series of subjects, narrative or systematic review, comments, or meta-analysis;
- If many studies have been carried out using duplicate cases, only the study with complete data and the largest sample size was included.

Definitions

SSc was defined according to the 2013 classification criteria.[11] The distinction between diffuse SSc (dSSc) and limited SSc (lSSc) was based upon LeRoy criteria.[12] Assessment of skin involvement was based on modified Rodnan skin score (mRSS).[13] Vit D insufficiency was defined as a Vit D level below 30 ng/mL, and Vit D deficiency as a Vit D level below 10 ng/mL. Interstitial lung disease (ILD) was defined on the basis of findings of high-resolution computed tomography. Pulmonary hypertension (PAH) was defined as a pulmonary arterial pressure > 35 mmHg in color Doppler echocardiography on at least two occasions.

Data extraction

Data were extracted using a predeveloped form and entered in an Excel datasheet. Two investigators (T.D. and I.S.)

independently extracted the following information: first author, year of publication, study design, country, ethnicity, mean or median age, gender ratio (M/F), number of patients, number of controls, frequencies of Scl70 Ab, ACA, dSSc, lSSc, ILD and pulmonary arterial hypertension (PAH), systolic pulmonary arterial pressure (sPAP), digital ulcers (DU), mRSS, digestive tract involvement (DTI), forced vital capacity (FVC), diffusing capacity of the lungs for carbon monoxide (DLCO), Valentini disease activity index, and Medsger severity scale (Table 1). A third investigator (A.R.) compared the results of the extracted data for potential discrepancies.

Quality assessment

The quality of eligible studies was assessed independently by two reviewers (T.D. and I.S.) using the Newcastle–Ottawa Scale (NOS)[14] which is based on the following three general categories: selection (4 points), comparability of the study groups (2 points), and ascertainment of outcome (3 points). Studies with a score $\geqslant 7$ were classified as high-quality reports. In addition, risk of bias was assessed for each included study through a generic form (Excel spreadsheet) and visualized via the Cochrane ROBVIS online tool (https://mcguinlu.shinyapps.io/robvis/). Two additional independent reviewers (T.B.A. and Y.G.) examined the quality-assessment results.

Study endpoints

The primary endpoint of this meta-analysis was to estimate the strength of the association of Vit D level and Vit D insufficiency/deficiency with susceptibility to SSc and its clinical features (dSSc vs lSSc, mRSS, ILD, sPAP, DU, DTI, and calcinosis). The secondary endpoint was to evaluate potential confounding factors that might influence the impact of the aforementioned associations in order to identify the sources of heterogeneity.

Statistical analysis

Statistical analysis was carried out using the Cochrane Review Manager 5.4 software, the OpenMeta-Analyst software, the StatsDirect software, and the Jamovi software. Pooled raw mean differences (RMD) with 95% confidence interval (95% CI) were computed to assess the association of Vit D levels with susceptibility to SSc, SSc subtypes, ILD, DU, DTI, and calcinosis; and mRSS, sPAP, and FVC levels with Vit D insufficiency. The associations of Vit D insufficiency or deficiency with SSc occurrence, ILD, and Scl70 Ab frequencies were assessed using pooled odds ratios (ORs) with the 95% CI. Pooled correlation between Vit D level and mRSS was estimated using Fisher's Z transformation. The statistical significance of pooled RMD, ORs, correlations was tested by Z-test with a threshold of significance set at 0.05. Random effects models (DerSimonian–Laird) were used as recommended by Borenstein et al.[15] Indeed, as long as the eligible studies were carried out in genetically diverse populations with disparate environmental exposures, dietary habits, Vit D intake, and sun exposure, the random-effects model applies.[15] Forest plots were generated to display the distribution of effect sizes (mean difference, OR, correlation *r*) across included studies. Sensitivity analyses were carried out to test the results stability by omitting sequentially each individual study. The heterogeneity of between-studies was tested by Q test (significance threshold: 0.1), quantified via I^2 calculation (proportion of true effects variance) and analyzed through the determination of 95% prediction intervals (PI). PI were obtained through the CMA Prediction Intervals free software. Calculation of the 95% PI was based on the following four items: effect size (mean difference, OR, or correlation *r*), upper bound of 95% CI, Tau,^{2} and number of included studies. Of note, 95% PI computation requires at least three included studies. Permission to use the CMA PI has been obtained since 21 March 2023 (Figure S1). Subsequently, the heterogeneity was explored for potential sources by subgroup analyses and meta-regressions. Briefly, studies were stratified by ethnicity (Caucasian, Asian, Middle-Eastern, and South American). Meta-regressions were performed using age, gender ratio (male/female), Scl70 Ab frequency, ACA frequency, dSSc/lSSc ratio, ILD, PAH, and mRSS. Both univariate and multivariate models of meta-regression were generated in order to assess the presence of potential confounding factors. Outlier identification and assessment were performed via the determination of (1) externally standardized residuals, (2) difference-in-fit standardized (DFFITS) statistic, (3) Cook's distances, (4) covariance ratios, (5) leave-one-out Tau^2 estimates, and (6) leave-one-out (residual) heterogeneity test statistic. Publication bias was assessed by Egger's test and visualized through the generation of funnel plots. Of note, Egger's regression requires at least three included studies.

Supplementary File 1 with additional tables and figures is available with the full-article. A PRISMA checklist is available as Supplementary File 2.

Systematic review registration

This review has been registered on PROSPERO: CRD4 2024565045, available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024565045.

Results

Search results and study characteristics

A PRISMA flow diagram was generated to depict the study selection process (Figure 1). Overall, 39 studies with a total of 2586 SSc cases and 1171 controls were included in the present study.[16–54] The characteristics of the included studies are recorded in Table 1. A total of 26

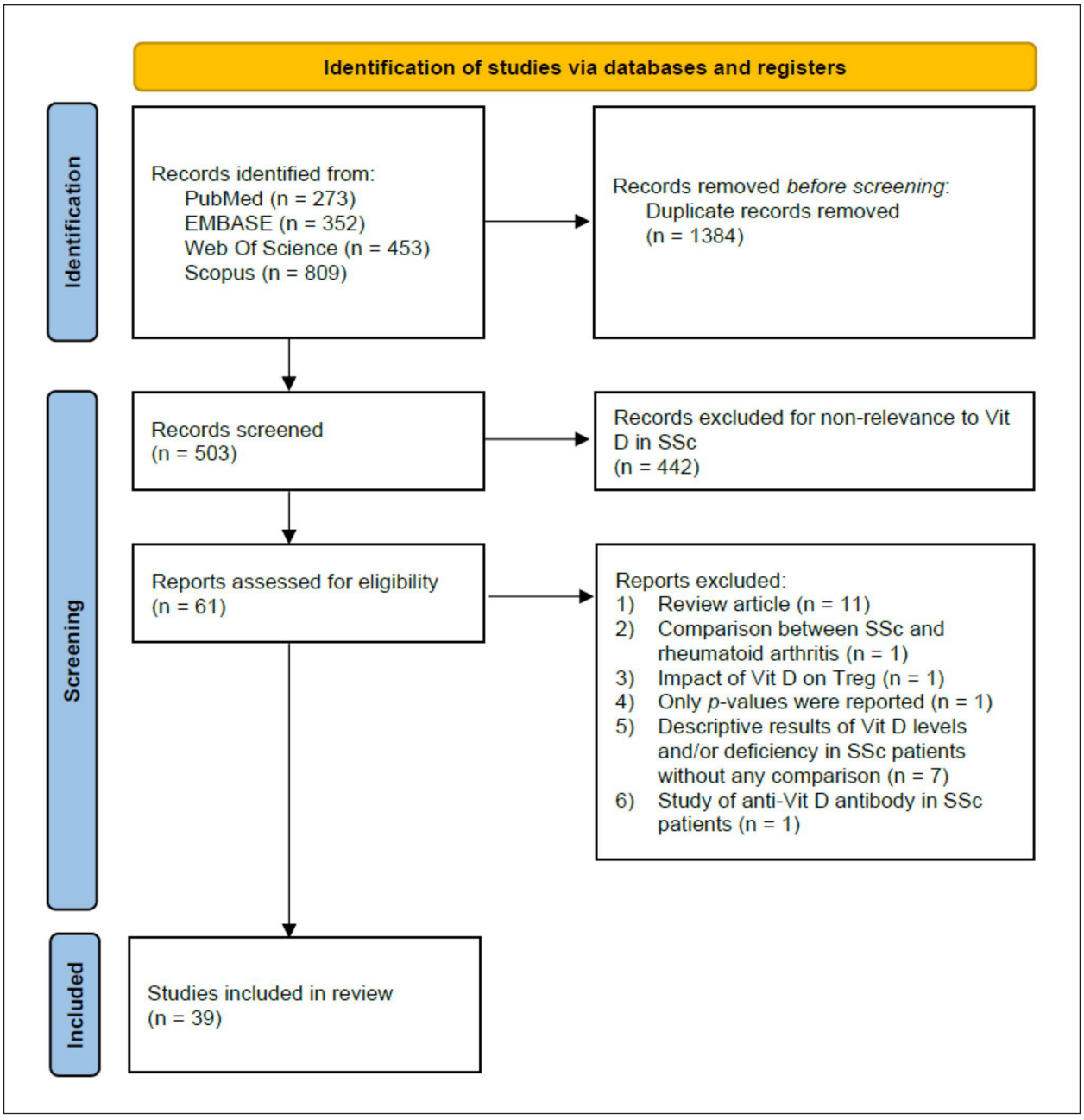

Figure 1. PRISMA flow diagram for study selection.

studies [16–19,21,24,25,28,31–43,46,48–50,54] with 30 distinct SSc groups were included for the association between Vit D level and susceptibility to SSc, 9 studies[19,24,28,32,33,36,43,46,54] for the association of Vit D insufficiency with SSc predisposition, 8 studies[19,24,28,32,34,43,46,54] for the association of Vit D deficiency with susceptibility to SSc, 9 studies[16,17,23,24,27,29,31,50,51] for the association between SSc subtypes and Vit D level, 5 studies[22,24,29,32,43] for the association of Vit D deficiency with mRSS, 7 studies [24,30,37,43,44,52,53] for the correlation of Vit D level with mRSS, 5 studies[17,22,44,51,53] for the association of Vit D level with ILD, 5 studies[22,29,43,53,54] for the association of Vit D deficiency with ILD, 4 studies[19,22,29,32] for the association of Vit D deficiency with sPAP, 4 studies[19,22,29,43] for the association of Vit D deficiency with DU, 5 studies[17,20,43,51,53] for the association of Vit D level with DU, 6 studies[17,30,31,44,51,53] for the association of Vit D level with DTI, 3 studies[26,45,47] for the association of Vit D level with calcinosis, 4 studies[19,22,43,54] for the association of Vit D insufficiency with Scl70 Ab, and 2 studies[32,53] for the association of Vit D insufficiency with FVC. Risks of bias are summarized in Figure 2.

Table 1. Study characteristics.

Ref.	Study	Country	Ethnicity	Age	Gender-ratio (M/F)	Scl70 Ab	ACA	dSSc	lSSc	ILD	PAH	mRSS	Patients (n)	Controls (n)	NOS Score
[16]	Ahmadi 2017	Iran	Asian	46.15 ± 9.63	0.132 (7/53)	56.7%	2%	50%	50%	56.7%	NS	NS	60	30	7
[17]	Arnson 2011	European	Caucasian	56.7 ± 18.2	0.143 (20/140)	24.1%	NS	28%	72%	61.1%	NS	NS	327	141	7
[18]	Atteritano 2013	Italy	Caucasian	54.43 ± 1.73	0/54	NS	NS	37%	63%	NS	NS	NS	54	54	8
[19]	Atteritano 2016	Italy	Caucasian	58.47 ± 14.04	0.053 (2/38)	35%	15%	70%	30%	44%	24%	10.45 ± 6.74	40	40	7
[20]	Caimmi 2019	Italy	Caucasian	58 ± 12	0.1607 (9/56)	26.2%	55.4%	30.8%	69.2%	23.1%	1.5%	9 ± 5	65	NA	7
[21]	Calzolari 2009	Italy	Caucasian	58 [31–72]	0.25 (12/48)	40%	40%	22%	78%	53%	57%	NS	60	60	6
[22]	Caramaschi 2010	Italy	Caucasian	58.1 ± 14.8	0.25 (13/52)	29.2%	53.8%	38.5%	61.5%	70.7%	3%	11 ± 5.4	65	NA	7
[23]	Corallo 2019	Italy	Caucasian	62	0.148 (8/54)	19%	42%	19.4%	80.6%	48%	32%	15 [3–29]	62	NA	7
[24]	Corrado 2015	Italy	Caucasian	64.5	0/64	51.6%	48.4%	48.4%	51.6%	70.3%	46.9%	17.4	64	35	8
[25]	Corrado 2022	Italy	Caucasian	57.6 ± 11.75	0.078 (4/47)	52.9%	37.25%	NS	NS	49%	13.7%	19.4 ± 9.2	51	31	7
[26]	Cruz-Domínguez 2017	Mexico	South American	51.4 ± 12	0.048 (5/104)	19.3%	28.4%	33.9%	66.1%	NS	NS	10.26	109	NA	6
[27]	Ece 2023	Turkey	Asian	50.02 ± 11.55	0.135 (7/52)	32.2%	44.1%	49.2%	50.8%	45.8%	16.9%	6.97	59	NA	5
[28]	El Gharbawy 2021	Egypt	Middle-Eastern	49.3 ± 4.3	NS	NS	NS	43.3%	56.6%	NS	NS	12.16 ± 3.75	30	20	6
[29]	Groseanu 2016	Romania	Caucasian	55.65 ± 12.45	NS	60.78%	17.64%	52.9%	47.1%	41.17%	NS	9.59 ± 5.01	51	NA	6
[30]	Gupta 2018	India	Asian	44.9 ± 12.13	0.151 (5/33)	65.8%	10.5%	63.15%	26.3%	76.3%	21%	NS	38	38	5
[31]	Hajialilo 2017	Iran	Asian	46.2 ± 9.7	0.111 (6/54)	60%	8.33%	70%	30%	NS	NS	NS	60	60	7
[32]	Hax 2020	Brazil	South American	57.2 ± 12.8	0.064 (3/47)	NS	NS	28%	72%	NS	10%	6 [2.75–10.25]	50	35	8
[33]	Horvàth 2019	Hungary	Caucasian	64.1 ± 9	0.222 (8/36)	25%	15.9%	25%	75%	79.5%	6.8%	NS	44	33	6
[34]	Ibn Yacoub 2012	Morocco	Middle-Eastern	49.44 ± 13.12	0/30	NS	NS	85%	15%	26.6%	NS	>15 in 48%	30	30	7
[35]	Isola 2021	Italy	Caucasian	53.2 ± 3.5	0.842 (16/19)	NS	NS	76.2%	11.9%	NS	NS	17.5 [16.4–18.3]	35	37	7
[35]	Isola 2021 (SSc + PO)	Italy	Caucasian	52.5 ± 2.9	0.714 (15/21)	NS	NS	73.2%	14.6%	NS	NS	18.9 [15.6–20.4]	36	37	7
[36]	Jud 2023	Austria	Caucasian	57.9 ± 9.2	0.055 (2/36)	NS	NS	0	100%	5.3%	0%	4.84 ± 3.84	38	38	6
[37]	Kotyla 2018	Poland	Caucasian	55 ± 19	0.6 (18/30)	62.5%	25%	100%	0	83.3%	16.7%	11.2 ± 7.3	48	23	7
[38]	Lee 2022	Korea	Asian	61.7 ± 7.7	0.112 (7/62)	20.3%	50.7%	46.4%	53.6%	65.2%	5.8%	7.71	69	38	7
[39]	Lo Gullo 2021	Italy	Caucasian	64.1 ± 12.5	0/36	25%	44.4%	66.7%	33.3%	47.2%	NS	28.9 ± 10.2	36	36	7
[40]	Montabone 2016	Italy	Caucasian	61	0.0937 (3/32)	NS	NS	NS	NS	NS	NS	NS	35	60	6
[41]	Omelchenko 2021	Ukraine	Caucasian	1–17	0.555 (5/9)	NS	NS	NS	NS	NS	NS	NS	14	10	5
[42]	Orbach 2007	Israel	Middle-Eastern	NS	NS	NS	NS	NS	NS	NS	NS	NS	229	30	5
[43]	Park 2017	Korea	Asian	60.9 ± 10.5	0/40	25%	32.5%	37.5%	62.5%	35%	10%	9.2 ± 5.4	40	80	8
[44]	Sampaio-Barros 2016	Brazil	South American	40.18 ± 7.27	0/38	50%	NS	100%	0%	79%	3%	6.55 ± 4.67	38	NA	7
[45]	Sampaio-Barros 2019	Brazil	South American	56.42 ± 9.125	0/72	22.2%	37.5%	0%	100%	40.27%	NS	2.725 ± 3.58	72	NA	7
[46]	Seriolo 2011	Italy	Caucasian	58.5 ± 7.3	0/53	NS	NS	NS	NS	NS	NS	NS	53	35	7
[47]	Serup 1985	Denmark	Caucasian	52.8	0.428 (6/14)	NS	NS	NS	NS	NS	NS	NS	25	NA	6
[48]	Shinjo 2011	Brazil	South American	20.9 ± 1.3	0.111 (1/9)	20%	10%	70%	30%	NS	NS	21.9 ± 15.5	10	10	6
[49]	Sirufo 2021	Italy	Caucasian	64.63	NS	NS	NS	NS	NS	NS	NS	NS	42	40	6
[50]	Taylan 2018	Turkey	Asian	53 ± 10.4	0/46	45.7%	39.1%	39.1%	60.9%	56%	6.5%	7	46	30	7
[51]	Trombetta 2017	Italy	Caucasian	59 ± 15	0.1756 (23/131)	28.2%	56.4%	25%	75%	46%	NS	NS	154	NA	7
[52]	Ursini 2017	Italy	Caucasian	59 ± 14.7	0.877 (10/114)	NS	NS	19.4%	80.6%	42.7%	17.7%	7.7 ± 1.4	124	NA	6
[53]	Zebryk 2021	Poland	Caucasian	53.8 ± 13.5	0.4655 (27/58)	NS	NS	29%	66%	78%	13%	13 ± 8	68	NA	7
[54]	Zhang 2017	China	Asian	47.75 ± 12.73	0.154 (8/52)	28.33%	NS	100%	0%	NS	NS	NS	60	60	7

ACA: Anti-centromere Ab; dSSc: diffuse SSc; lSSc: limited SSc; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; mRSS: modified Rodnan skin score; NS: not specified; NA: not applicable; PO: periodontitis.

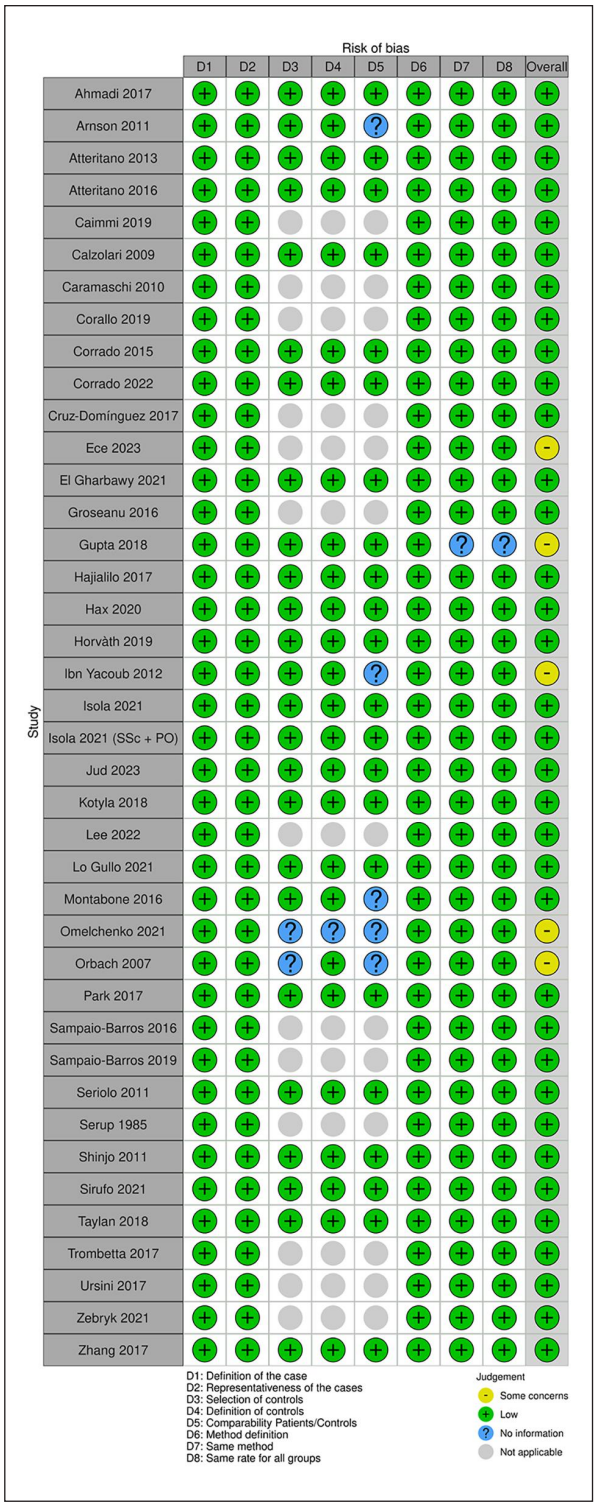

Figure 2. Summary of study risk of bias.

Vitamin D level in SSc patients and healthy controls

Combined analysis revealed a significant decrease in Vit D level in SSc patients comparatively to healthy subjects, RMD = −11.68 [−15.43 to −7.92] ng/mL, $p > 1$ E-10 (Table 2 and Figure 3). However, there was a very high level of between-studies heterogeneity, $I^2 = 98\%$, $p > 1$ E-10, $Tau^2 = 104.64$, 95% PI = −33 to 9.64 ng/mL. Interestingly, outlier analysis revealed that the study by Ibn Yacoub 2012[34] was a significantly outlying study (Figure 4). Indeed, all outlier indices (externally standardized residuals, difference-in-fit standardized statistic, Cook's distances, covariance ratios, leave-one-out Tau^2 estimates, and leave-one-out heterogeneity test statistic) showed that the Ibn Yacoub 2012 study, in which the reported decrease in Vit D was −46.53 ng/dL, contributed significantly to the between-studies heterogeneity. Hence, omitting the Ibn Yacoub 2012 study resulted in an almost fivefold decrease in Tau^2 (variance of true effects) from 104.64 to 22.88. After omitting the Ibn Yacoub 2012 study, RMD was −10.29 [−12.21 to −8.37], I^2 decreased to 92%, and the 95% PI was [−20.31 to −0.27], which excluded the zero value (Table 2). Subsequent subgroup analysis by ethnicity did not reveal any inter-ethnic significant difference (Tables S1 and S2) (Figure S2). Conversely, meta-regressions revealed significant correlations of the effect size (mean difference) with Scl70 Ab frequency, dSSc/lSSc ratio, and PAH frequency; $p = 0.0001$, $p = 0.02$, and $p = 0.024$, respectively (Table S2 and Figure S3).

Vit D insufficiency association with susceptibility to SSc

Integrated analysis revealed a significant association between Vit D insufficiency (Vit D < 30 ng/mL) and susceptibility to SSc, OR 95% CI = 3.58 [2.59–4.95], $p < 1$ E-10 (Table 2 and Figure 5). Of note, there was no heterogeneity between included studies, $I^2 = 0\%$, $p = 0.72$, $Tau^2 = 0$, 95% PI = [2.59–4.95]. Subgroup analysis by ethnicity showed that the association of Vit D insufficiency with SSc risk remained stable in all ethnic groups (Tables S3 and S4, and Figure S4). Likewise, meta-regression revealed no significant correlation of the effect size with the clinical and biological features of SSc patients (Table S4).

Vit D deficiency association with susceptibility to SSc

Vit D deficiency (Vit D < 10 ng/mL) was significantly associated with SSc, OR 95% CI = 7.67 [3.97–14.83], $p < 1$ E-10 (Table 2 and Figure 6). No between-studies heterogeneity was observed, $I^2 = 0\%$, $p = 0.4998$, $Tau^2 = 0$, 95% PI = [3.97–14.83]. Subgroup analysis revealed no inter-ethnic significant difference regarding the association of Vit D deficiency with susceptibility to SSc (Tables S5 and S6, and Figure S5). Similarly, meta-regression showed no correlation of the effect size with clinical and biological parameters in SSc patients (Table S6).

Table 2. Main results for the Vit D associations with SSc and its clinical and biological features.

SSc vs controls	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D level (ng/mL)	−11.68 [−15.43 to −7.92]	<1 E-10	98%	<1 E-10	104.64	−33 to 9.64
Omitting Ibn Yacoub 2012	−10.29 [−12.21 to −8.37]	<1 E-10	92%	<1 E-10	22.88	−20.31 to −0.27
SSc vs controls	OR [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D < 30 ng/mL	3.58 [2.59 to 4.95]	<1 E-10	0%	0.72	0	2.59 to 4.95
SSc vs controls	OR [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D < 10 ng/mL	7.67 [3.97 to 14.83]	<1 E-10	0%	0.4998	0	3.97 to 14.83
dSSc vs lSSc	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D level (ng/mL)	−2.26 [−4.87 to 0.35]	0.09	88%	<1 E-10	12.54	−11.29 to 6.69
Vit D < 10 vs ⩾ 10 ng/mL	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
mRSS	2.08 [−0.92 to 5.08]	0.175	78%	0.001	8.89	−8.59 to 12.75
mRSS vs Vit D level	Pearson *r* [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
	−0.26 [−0.44 to −0.08]	0.004	77%	5.2 E-5	0.0419	−0.69 to 0.3
Omitting Corrado 2015	−0.19 [−0.28 to −0.1]	0.0001	0%	0.511	0	-0.28 – -0.1
ILD vs no-ILD	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D level (ng/mL)	−3.61 [−6.93 to −0.3]	0.03	47%	0.11	6.43	−13.88 to 6.66
Vit D < 10 vs ⩾ 10 ng/mL	OR [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
ILD	1.84 [0.66 to 5.14]	0.24	53%	0.0757	0.701	0.08 to 42.66
Vit D < 10 vs ⩾ 10 ng/mL	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
sPAP (mmHg)	4.17 [1.44 to 6.89]	0.003	20%	0.288	1.768	0.13 to 8.21
Vit D < 10 vs ⩾ 10 ng/mL	OR [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
DU	2.15 [0.83 to 5.59]	0.115	47%	0.13	0.4469	0.15 to 29.92
DU vs no-DU	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D level (ng/mL)	−3.46 [−10.13 to 3.22]	0.31	83%	0.0001	43.34	−27.05 to 20.13
DTI vs no-DTI	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D level (ng/mL)	−0.07 [−2.84 to 2.70]	0.962	70%	0.005	7.81	−8.76 to 8.62
Calcinosis	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Vit D level (ng/mL)	4.18 [1.07 to 7.28]	0.008	0%	0.71	0	1.07 to 7.28
Vit D < 30 vs ⩾ 30 ng/mL	OR [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
Scl70 Ab+	2.62 [1.33 to 5.16]	0.005	0%	0.49	0	1.33 to 5.16
Vit D < 30 vs ⩾ 30 ng/mL	Mean difference [95% CI]	*p*-value	I^2	*p*-value	Tau^2	95% PI
FVC	−6.02 [−11.9 to −0.15]	0.04	0%	0.59	0	−11.9 to −0.15

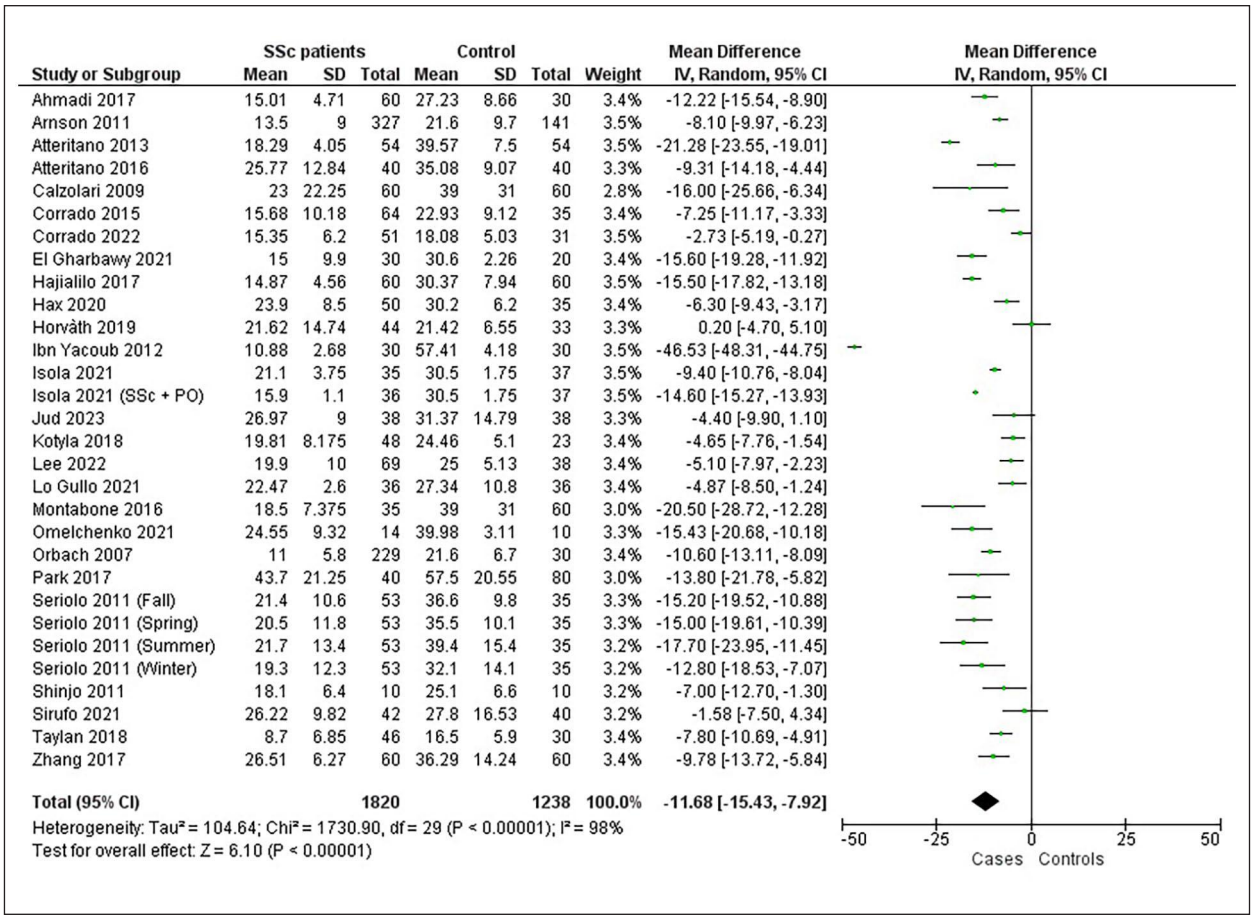

Figure 3. Forest plot for the association between Vit D level and susceptibility to SSc.

Vit D level association with SSc subsets (dSSc vs lSSc)

Joint analysis showed a trend toward lower Vit D levels in dSSc patients when compared with lSSc cases, but the difference did not reach statistical significance, RMD = −2.26 [−4.87 to 0.35] ng/mL, $p = 0.09$ (Table 2 and Figure 7). Nevertheless, there was a large amount of between-studies heterogeneity, $I^2 = 88\%$, $p < 1$ E-10, $Tau^2 = 12.54$, 95% PI = [−11.29 to 6.69]. Subgroup analysis by ethnicity yielded no significant difference regarding the effect size between Asians and Caucasians (Tables S7 and S8, and Figure S6). Conversely, meta-regression showed that the effect size was positively correlated with gender-ratio (M/F) $p < 1$ E-10, and negatively correlated with ILD frequency in SSc patients, $p = 3.9$ E-5 (Figure S8).

Vit D deficiency association with mRSS

Combined analysis yielded a nonsignificant increase of mRSS in the case of Vit D deficiency, RMD: 2.08 [−0.t to 5.08], $p = 0.175$ (Table 2 and Figure 8). However, there was a high level of between-studies heterogeneity, $I^2 = 78\%$, $p = 0.001$, $Tau^2 = 8.89$, 95% PI = [−8.59 to 12.75]. Subsequent subgroup by ethnicity analysis showed stable results between the different ethnic groups (Tables S9 and S1, and Figure S8). Inversely, meta-regression revealed a significant positive correlation of the effect size with patients' age, $p = 0.0006$ (Table S10 and Figure S9). Hence, increase of mRSS in the case of Vit D deficiency was found to be larger in older SSc patients.

Correlation between Vit D level and mRSS

Integrated analysis revealed a significant negative correlation of the Vit D level with mRSS, Pearson $r = -026$ [−0.44 to −0.08], $p = 0.004$ (Table 2 and Figure 9). Nonetheless, there was a huge amount of between-studies heterogeneity, $I^2 = 77\%$, $p = 5.2$ E-5, $Tau^2 = 0.0419$, 95% PI = [−0.69 to 0.3]. Remarkably, outlier analysis revealed that the study by Corrado 2015,[24] in which Pearson r was −0.7, was the source of the entire between-studies heterogeneity (Figure 10). Indeed, omitting the study by Corrado 2015 reduced Tau^2 to zero (Table 2). Therefore, and after removing the study by Corrado 2015, the pooled r was −0.19 [−0.28 to −0.1], $p = 0.0001$, and $I^2 = 0\%$. Besides, subgroup by ethnicity analysis exhibited no inter-ethnic significant difference (Tables S11 and S12, and Figure S10). Conversely,

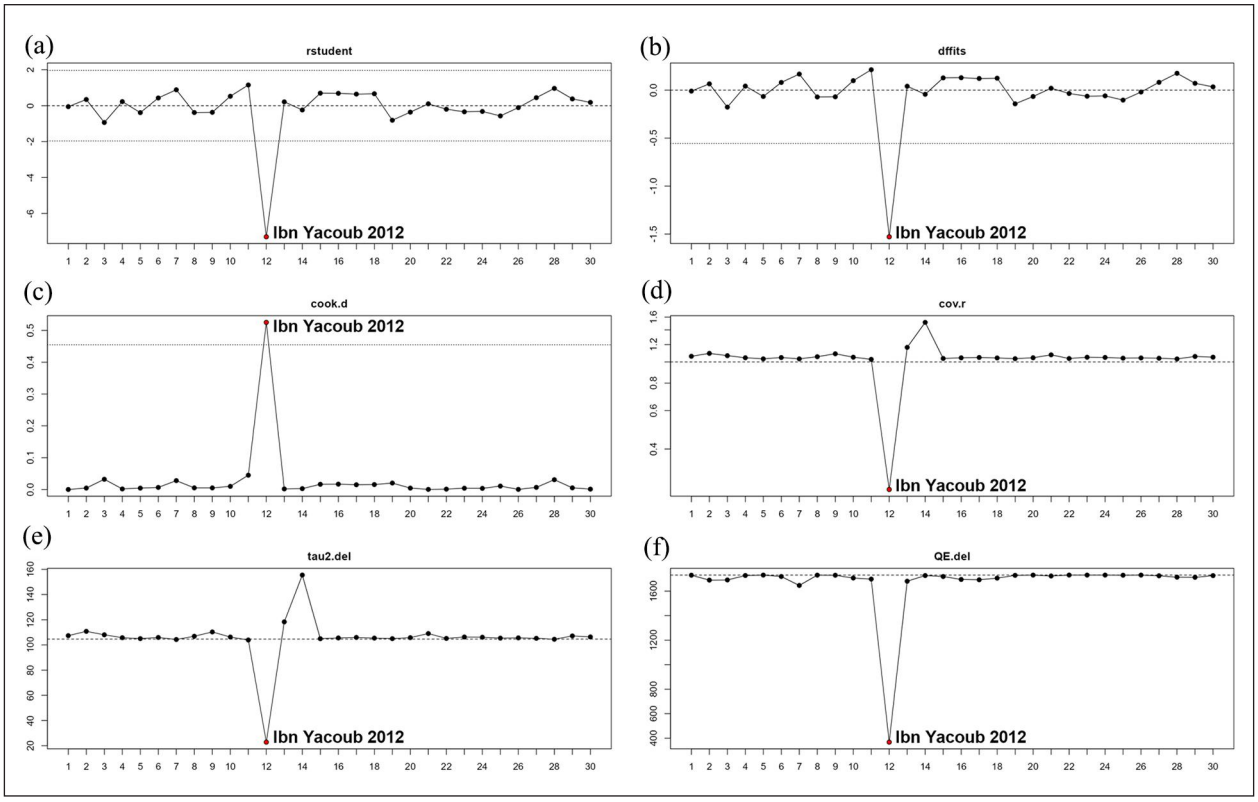

Figure 4. Outlier analysis for the association of Vit D level with susceptibility to SSc: (a) externally standardized residuals; (b) difference-in-fit standardized (DFFITS) statistic; (c) Cook's distances; (d) Covariance ratios; (e) leave-one-out Tau^2 estimates; and (f) leave-one-out (residual) heterogeneity test statistic.

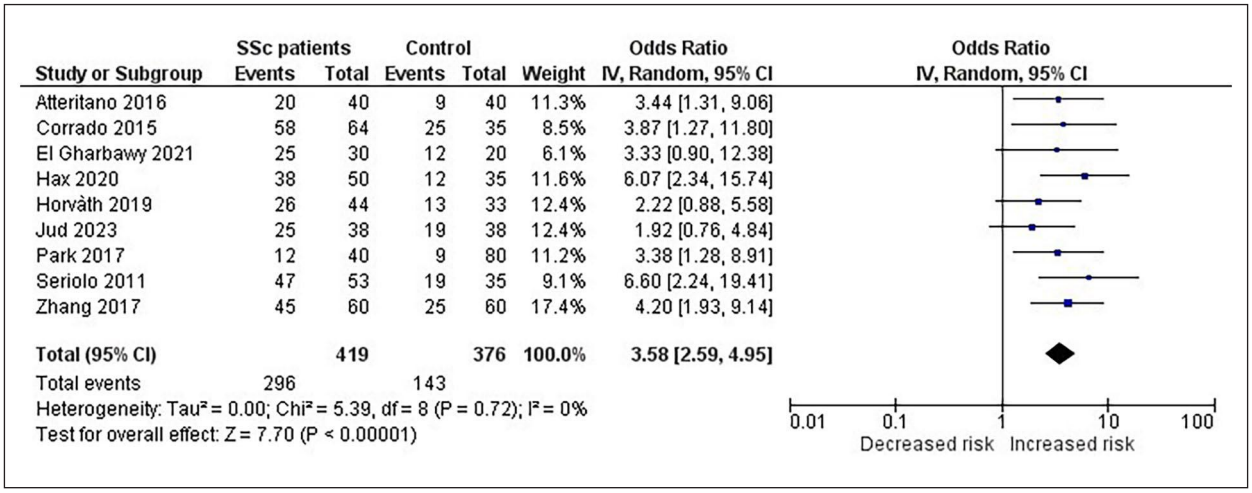

Figure 5. Forest plot for the association between Vit D insufficiency and SSc predisposition.

meta-regression showed significant negative correlations of the effect size with patients' age ($p=0.025$), and ACA frequency ($p=0.001$); see Table S12 and Figure S11. Hence, since the mean effect size was a negative correlation, the strength of the association was greater in older patients and in those with ACA.

Association of Vit D level with ILD

Only five studies investigated Vit D level in patients with or without ILD. Vit D level was found to be significantly decreased in patients with ILD, RMD 95% CI=−3.61 [−6.93 to −0.3] ng/mL, $p=1$ E-10 (Table 2 and Figure 11).

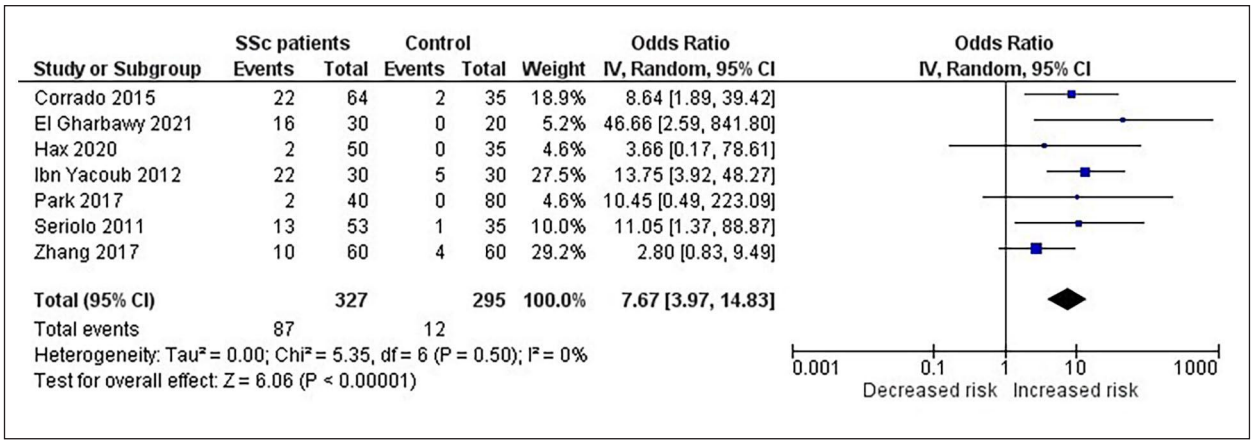

Figure 6. Forest plot for the association between Vit D deficiency and SSc predisposition.

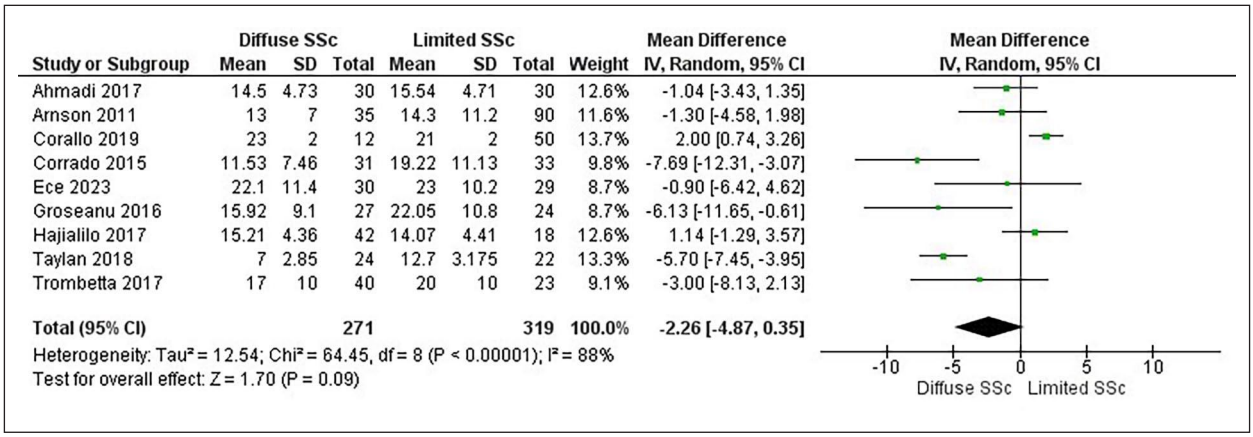

Figure 7. Forest plot for the association between Vit D level and SSc subtypes.

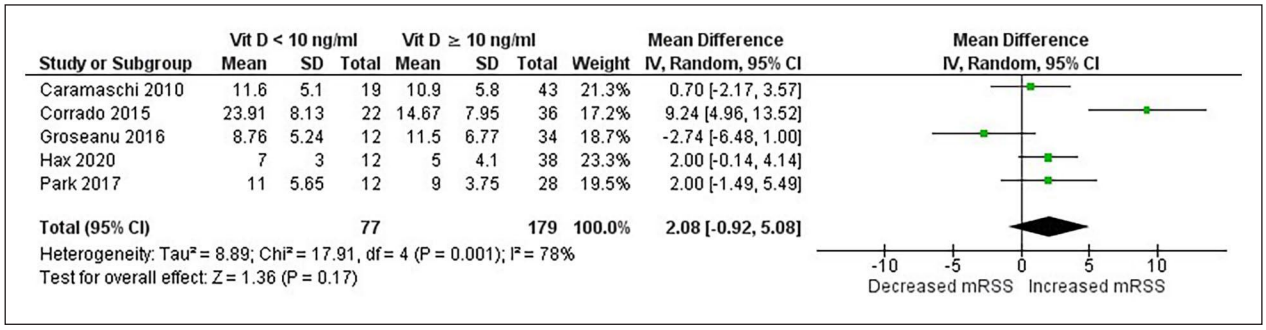

Figure 8. Forest plot for the association between Vit D deficiency and mRSS

However, there was a moderate amount of between-studies heterogeneity, $I^2=47\%$, $p=0.11$, $Tau^2=6.43$, 95% PI=[−13.88 to 6.66]. Subgroup analysis by ethnicity was not informative as the majority of included studies were performed in Caucasian groups along with only one study from South America (Tables S13 and S14, and Figure S12). Likewise, meta-regression showed no correlation of the effect size with SSc clinical and biological features (Table S14).

Association of Vit D deficiency with ILD

Yet again, only five studies examined the association of Vit D deficiency with ILD occurrence. Joint analysis revealed no significant association (OR 95% CI=1.84 [0.66–5.14], $p=0.24$), though with a significant between-studies heterogeneity, $I^2=53\%$, $p=0.0757$, $Tau^2=0.701$, 95% PI=[0.08–42.66]; see Table 2 and Figure 12. Subgroup by ethnicity analysis and meta-regressions were performed in order to

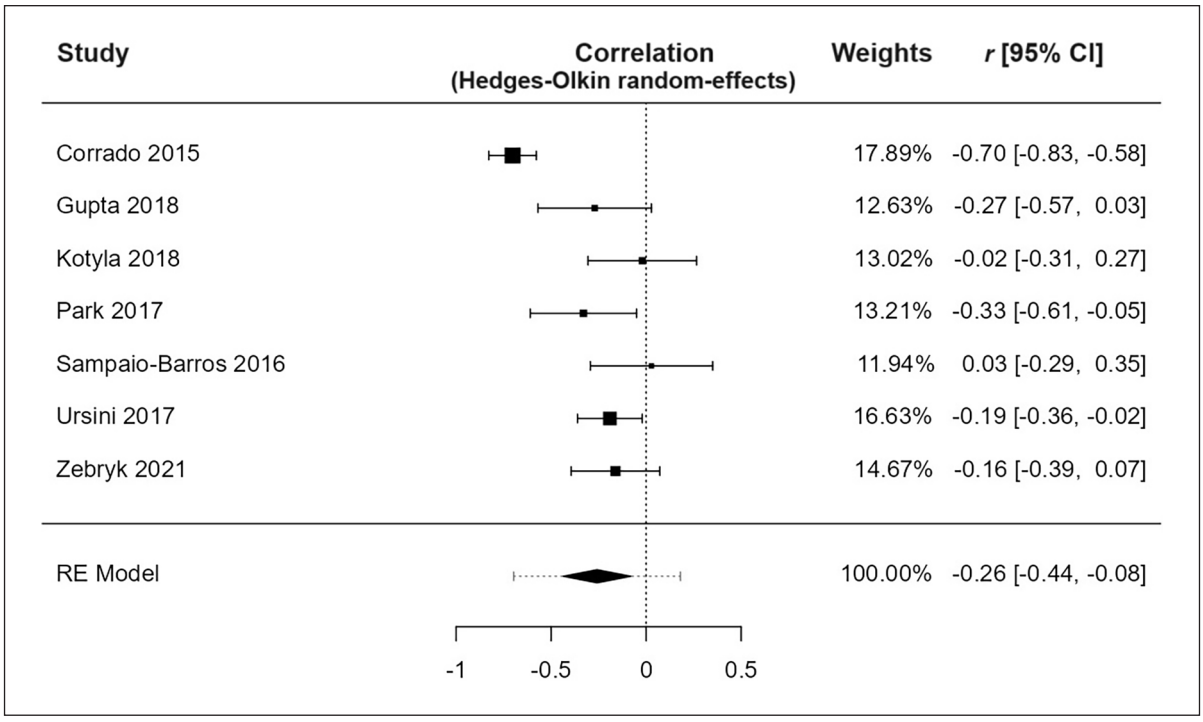

Figure 9. Forest plot for the correlation between Vit D level and mRSS.

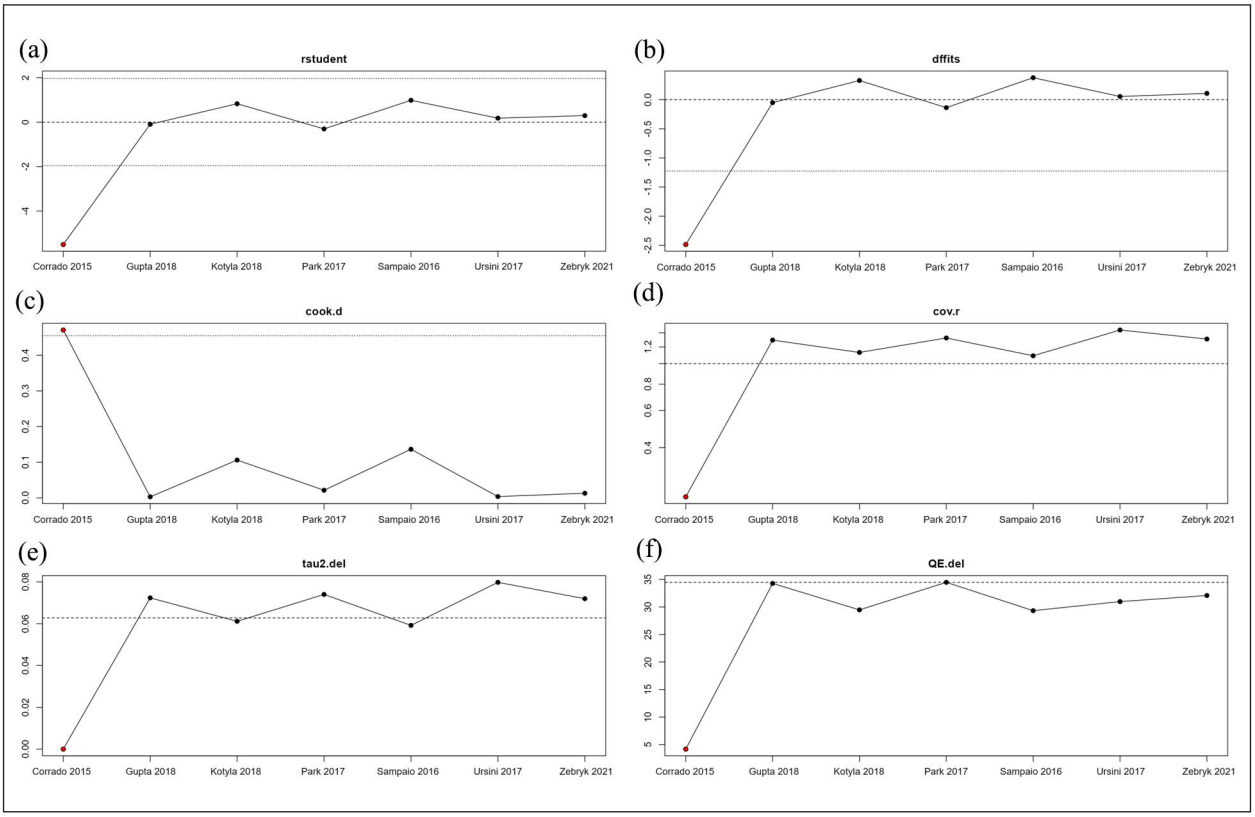

Figure 10. Outlier analysis for the correlation of Vit D level with mRSS: (a) externally standardized residuals; (b) difference-in-fit standardized (DFFITS) statistic; (c) Cook's distances; (d) covariance ratios; (e) leave-one-out Tau^2 estimates; and (f) leave-one-out (residual) heterogeneity test statistic.

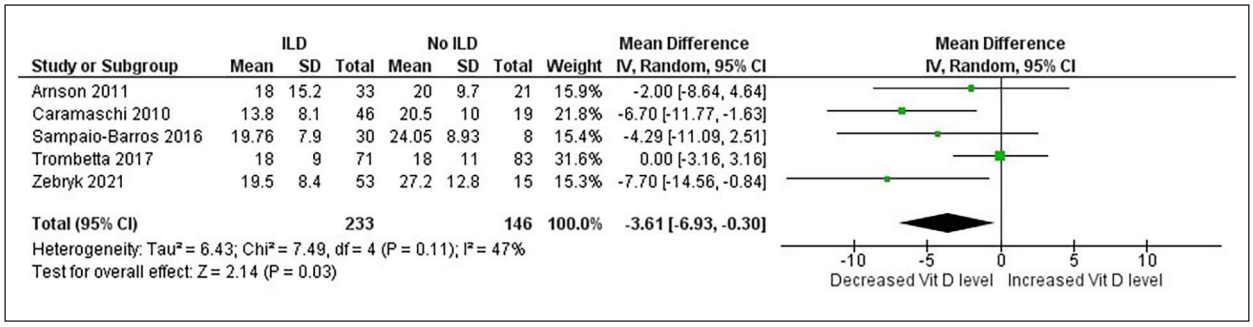

Figure 11. Forest plot for the association between Vit D level and ILD.

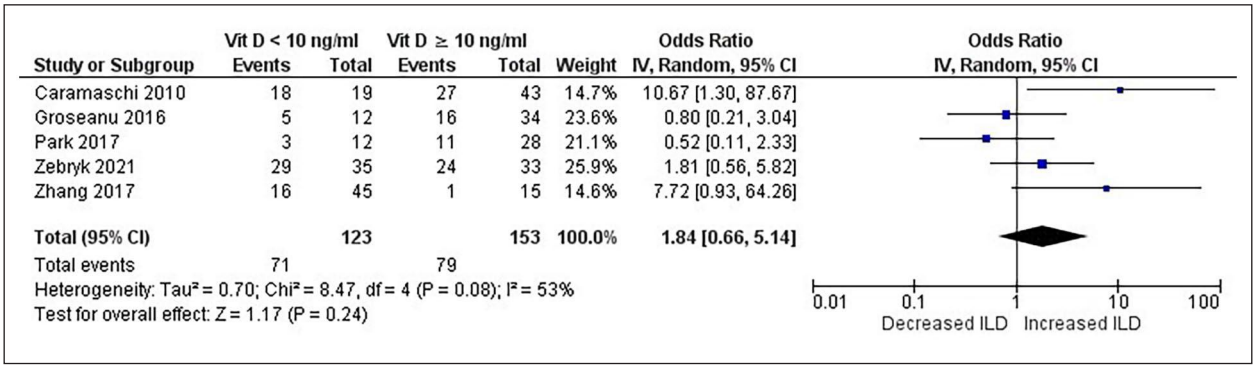

Figure 12. Forest plot for the association between Vit D deficiency and ILD.

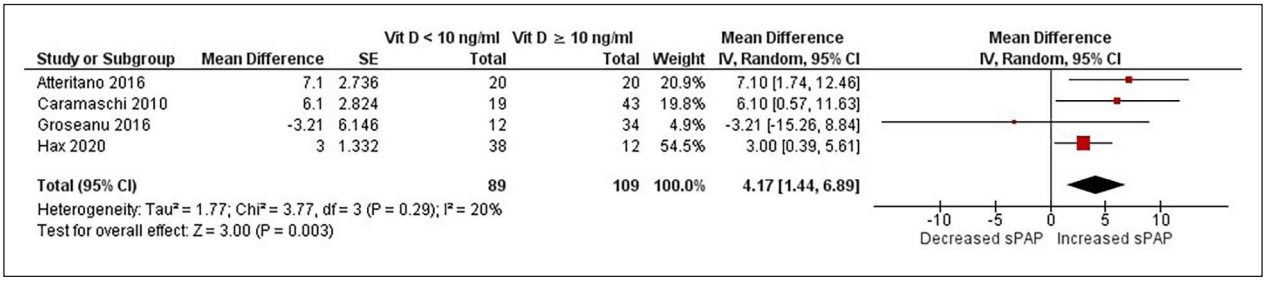

Figure 13. Forest plot for the association between Vit D deficiency and sPAP.

explore potential sources of heterogeneity, but did not reveal any significant difference or correlation (Tables 15 and 16, and Figure S13).

Association of Vit D deficiency with sPAP

Only four studies investigated the influence of Vit D deficiency on sPAP. Combined analysis showed a significant increased sPAP in the case of Vit D deficiency, RMD 95% CI = 4.17 [1.44–6.89] mmHg, $p = 0.003$ (Table 2 and Figure 13). In addition, there was a low level of heterogeneity between included studies, $I^2 = 20\%$, $p = 0.288$, $\text{Tau}^2 = 1.768$, 95% PI = [0.13–8.21]. Subgroup by ethnicity analysis did not reveal any significant difference between Caucasians and South Americans (Tables S17 and S18, and Figure S14). Conversely meta-regression showed a significant positive correlation of patients' age with increased sPAP magnitude in Vit D deficiency, $p = 0.032$ (Table S18 and Figure S15).

Association of Vit D deficiency with digital ulcers

Combined analysis did not show any significant association between Vit D deficiency and DU occurrence (OR 95% CI = 2.15 [0.83–5.59], $p = 0.115$), yet with a moderate amount of between-studies heterogeneity, $I^2 = 47\%$, $p = 0.13$, $\text{Tau}^2 = 0.4469$, 95% PI = [0.15–29.92]; see Table 2 and Figure 14. Subgroup by ethnicity analysis revealed that while no significant association was observed in Caucasians

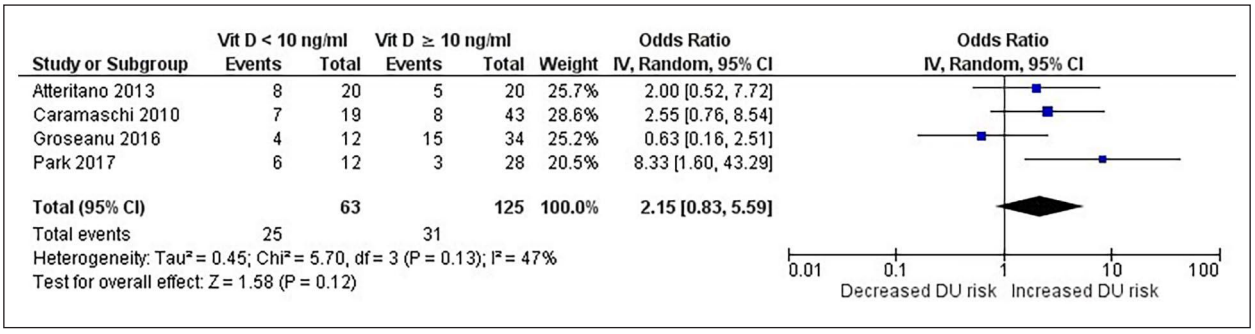

Figure 14. Forest plot for the association between Vit D deficiency and DU.

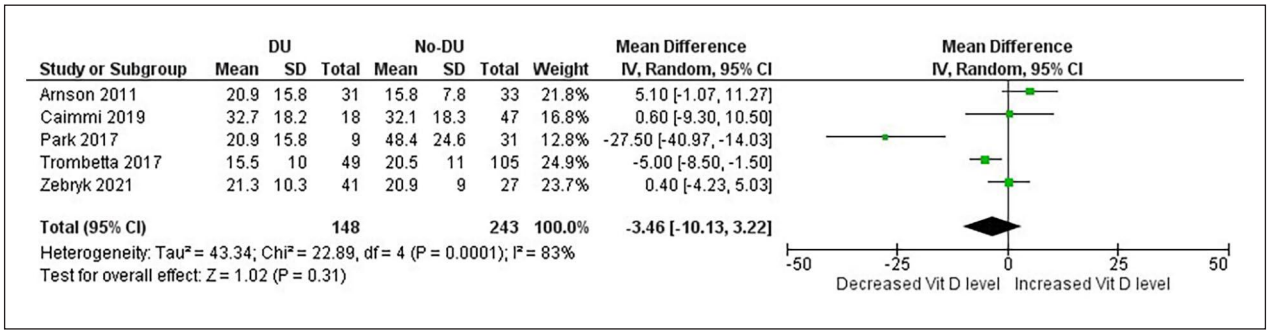

Figure 15. Forest plot for the association between Vit D level and DU.

($p=0.304$), Vit D deficiency increased DU occurrence in Asians, $p=0.011$ (Tables S19 and S20, and Figure S16). Moreover, meta-regression confirmed the inter-ethnic difference between Caucasians and Asians, and showed significant positive correlations of the effect size with gender-ratio (M/F), ILD frequency, and mRSS (Table S20 and Figure S17).

Association of Vit D level with digital ulcers

Five studies compared Vit D level between patients with or without DU. The decrease in Vit D level in the case of DU occurrence did not reach the statistical significance, RMD=−3.46 [−10.13 to 3.22], $p=0.31$ (Table 2 and Figure 15). Nevertheless, there was a high level of between-studies heterogeneity, $I^2=83\%$, $p=0.0001$, $Tau^2=43.34$, 95% PI=[−27.05 to 20.13]. Subgroup analysis showed a significant association between DU and Vit D level decrease in Asians (−27.5 [−40.98 to −14.03), $p=6.3$ E-5), whereas no association was noted in Caucasians (−0.22 [−4.98 to 4.54], $p=0.93$); see Table S21 and Figure S18. Meta-regression confirmed the inter-ethnic disparity regarding the association of Vit D level with DU occurrence, $p<0.0001$ (Table S22). Besides, the effect size (decreased Vit D level in DU) was found to be significantly and negatively correlated with patients' age and dSSc/lSSc ratio, and positively correlated with ACA frequency (Table S22 and Figure S19). Thus, since the mean effect size was negative, the association of decreased Vit D level with DU was rather noted in older patients and those with dSSc subset.

Vit D level association with digestive tract involvement

Joint analysis showed no difference in Vit D level between patients with or without DTI, RMD=−0.07 [−2.84 to 2.70] ng/mL, $p=0.962$ (Table 2 and Figure 16). However, there was a substantial level of between-studies, $I^2=70\%$, $p=0.005$, $Tau^2=7.81$, 95% PI=[−8.76 to 8.62]. Subgroup analysis exhibited no evidence of inter-ethnic disparity (Tables S23 and S24, and Figure S20). Similarly, meta-regression did not reveal any correlation of the effect size with clinical and biological features of SSc (Table S24).

Vit D level association with calcinosis

Only three studies examined Vit D level in patients with and without calcinosis. Vit D level was found to be significantly increased in patients with calcinosis, RMD=4.18 [1.07–7.28] ng/mL, $p=0.008$ (Table 2 and Figure 17). Of note, there was no heterogeneity between included studies, $I^2=0\%$, $p=0.71$, $Tau^2=0$, 95% PI=[1.07–7.28]. Meta-regression did not show any significant correlation of the effect size with patient age and gender-ratio (M/F) (Table S25).

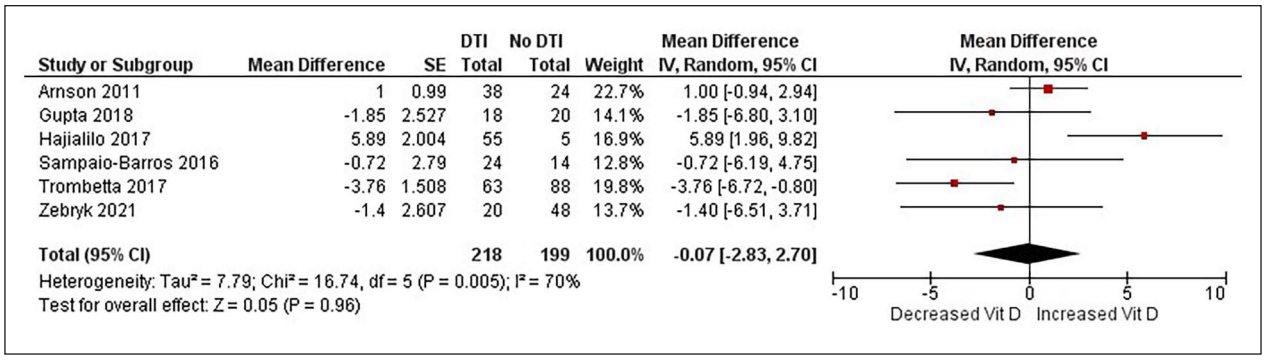

Figure 16. Forest plot for the association between Vit D level and DTI.

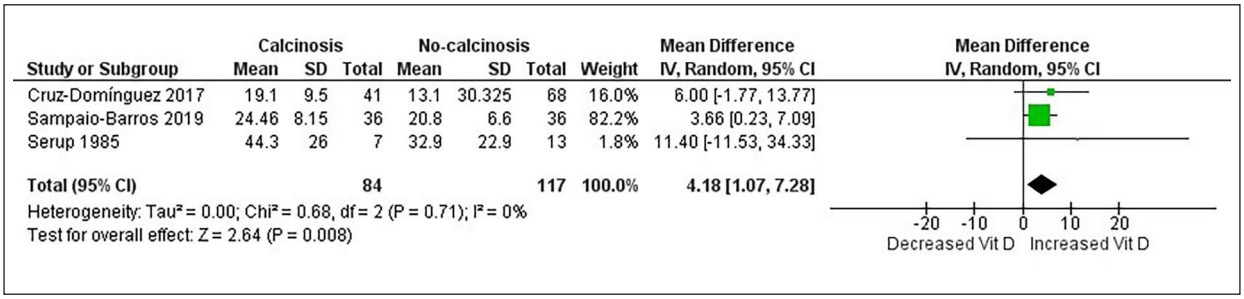

Figure 17. Forest plot for the association between Vit D level and calcinosis.

Association between Vit D insufficiency and Scl70 Ab

Vit D insufficiency was significantly associated with Scl70 Ab frequency (OR 95% CI = 2.62 [1.33–5.16], $p = 0.005$) with no evidence of between-studies heterogeneity, $I^2 = 0\%$, $p = 0.49$, $Tau^2 = 0$, 95% PI = [1.33–5.16]; see Table 2 and Figure 18. Subgroup analysis did not show any difference between Caucasians and Asians (Tables S26 and S27, and Figure S21). Subsequent meta-regression revealed a significant positive correlation of the effect size with patients age, $p = 0.008$ (Table S27 and Figure S22).

Association between Vit D insufficiency and forced vital capacity

Only two studies reported FVC estimation in patients with or without Vit D insufficiency. Combined analysis showed a significantly decreased FVC in the case of Vit D insufficiency, RMD = −6.02 [−11.9 to −0.15], $p = 0.04$ (Figure 19). Of note, there was no evidence of heterogeneity between included studies, $I^2 = 0\%$, $p = 0.59$, $Tau^2 = 0$, 95% PI = [−11 to −0.15]. As there were only two included studies, subgroup analysis and meta-regression could not be performed.

Sensitivity analysis

The sensitivity analysis revealed that association results were stable for all performed meta-analyses (Figures S23, S24, S25, S26, S27, S28, S29, S30, S31, S32, S33, S34, S35, and S36), suggesting a high level of integrity with reliable results.

Publication bias

Generated funnel plots (Figure 20) were found to be overall symmetrical and Egger's tests confirmed these findings with nonsignificant p-values for all performed comparisons in the current study (0.847, 0.7773, 0.4451, 0.2582, 0.6747, 0.8913, 0.3246, 0.2236, 0.9437, 0.5638, 0.7706, 0.8291, 0.0575, and 0.2761), which indicated that results were not weakened by publication biases.

Overall, decrease in Vit D was found to be associated with SSc susceptibility, increased mRSS, ILD occurrence, increased sPAP, decreased FVC, and Scl70 Ab presence. Conversely, calcinosis was associated with a higher level of Vit D.

Discussion

Vit D is an essential steroid hormone involved in skeletal health, cardiovascular function, and mineral metabolism.[7–9]

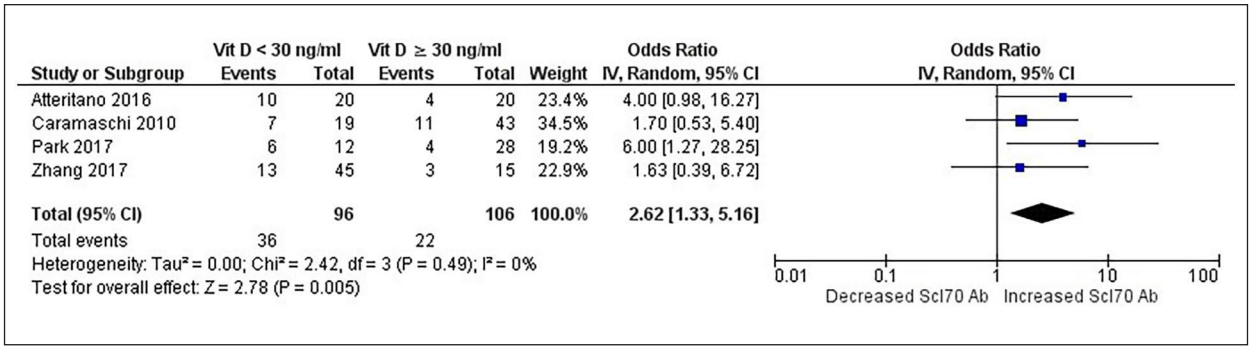

Figure 18. Forest plot for the association between Vit D insufficiency and Scl70 Ab.

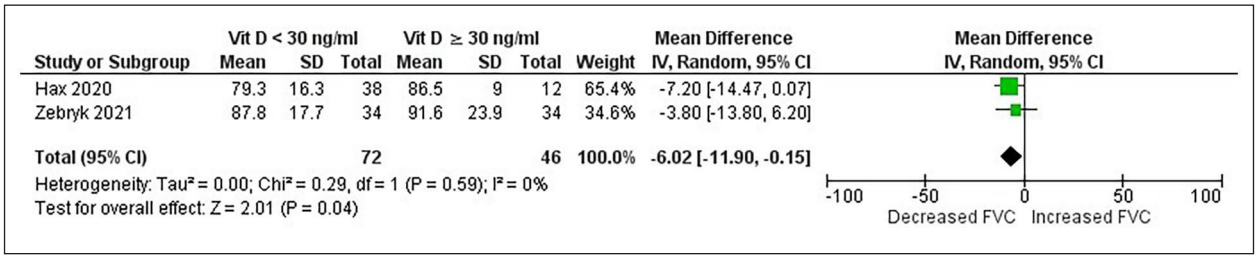

Figure 19. Forest plot for the association between Vit D insufficiency and FVC.

There is no consensus regarding the normal level of Vit D and the definition of the threshold for Vit D deficiency. Nevertheless, Vit D level below 30 ng/mL is widely considered as insufficient, whereas vitamin deficiency is defined disparately, with levels ranging from <10 to <20 ng/mL.

Over the past three decades, there has been accumulating evidence of Vit D's non-classical roles, including its immunomodulatory effects.[7–9] Indeed, although Vit D insufficiency/deficiency is common in healthy subjects, several observational studies and meta-analyses have reported significant associations between low Vit D levels and the risk of autoimmune diseases, including systemic lupus erythematosus (SLE),[55] rheumatoid arthritis (RA),[56] and multiple sclerosis (MS).[57] Moreover, Vit D levels were found to be associated with disease activity and/or severity of SLE,[58] RA,[56] Sjögren syndrome,[59] and MS.[60] Therefore, we sought in the present study to investigate the associations of Vit D with susceptibility to SSc and disease presentation. Although a previous meta-analysis was published in 2017,[61] the literature search cut-off time in our study was fresher. Moreover, and in addition to the fact that it included only eight studies, the meta-analysis of An et al.[61] has some shortcomings: (1) it missed some studies published long before,[21,34] (2) the authors used the fixed-effect model for the mean difference in Vit D level between SSc patients and controls while they should have applied the random-effects model, (3) the authors did not provide the 95% PI for true effects dispersion, and (4) the authors did not examine the sources of heterogeneity through subgroup analyses and meta-regressions. Thus, to the best of our knowledge, the present study was the first to explore the sources of between-studies inconsistency.

In this study, Vit D level was found to be significantly decreased in SSc patients when compared with healthy subjects, −10.29 [−12.21 to −8.37] ng/mL. However, there was a considerable heterogeneity between included studies, and the decrease in Vit D level could vary between −20.31 ng/mL and −0.27 ng/mL in 95% of comparable populations. Besides, while there was no inter-ethnic significant difference, the effect size was found to be significantly correlated with Scl70 Ab frequency, dSSc/lSSc ratio, and PAH frequency. Hence the decrease in Vit D level was larger in patients with Scl70 Ab, those with dSSc subset, and in the case of PAH occurrence. Furthermore, Vit D insufficiency conferred an approximately 3.58-fold increased SSc susceptibility, whereas Vit D deficiency was associated with about 7.67-fold increase in susceptibility to SSc. In contrast to Vit D level pooled effect, which exhibited a high level of between-studies heterogeneity, there was no apparent inconsistency in the association of Vit D insufficiency/deficiency with SSc occurrence. Taken together, these data indicated that the relationship between Vit D and SSc susceptibility was not linear, but rather observed when Vit D levels fall below a certain threshold.

In the present study, Vit D level decrease in dSSc subset comparatively to lSSc subset did not reach statistical significance (mean difference = −2.26 [−4.87–0.35] ng/mL, $p = 0.09$), though with a high level of inconsistency ($I^2 = 88\%$) between included studies. Thus, the effect size could fall between −11.29 ng/mL decrease and 6.69 ng/mL increase in 95% of comparable populations. While subgroup by ethnicity analysis was not informative, meta-regression exhibited significant correlations of the effect size with gender-ratio (M/F) and ILD occurrence. This

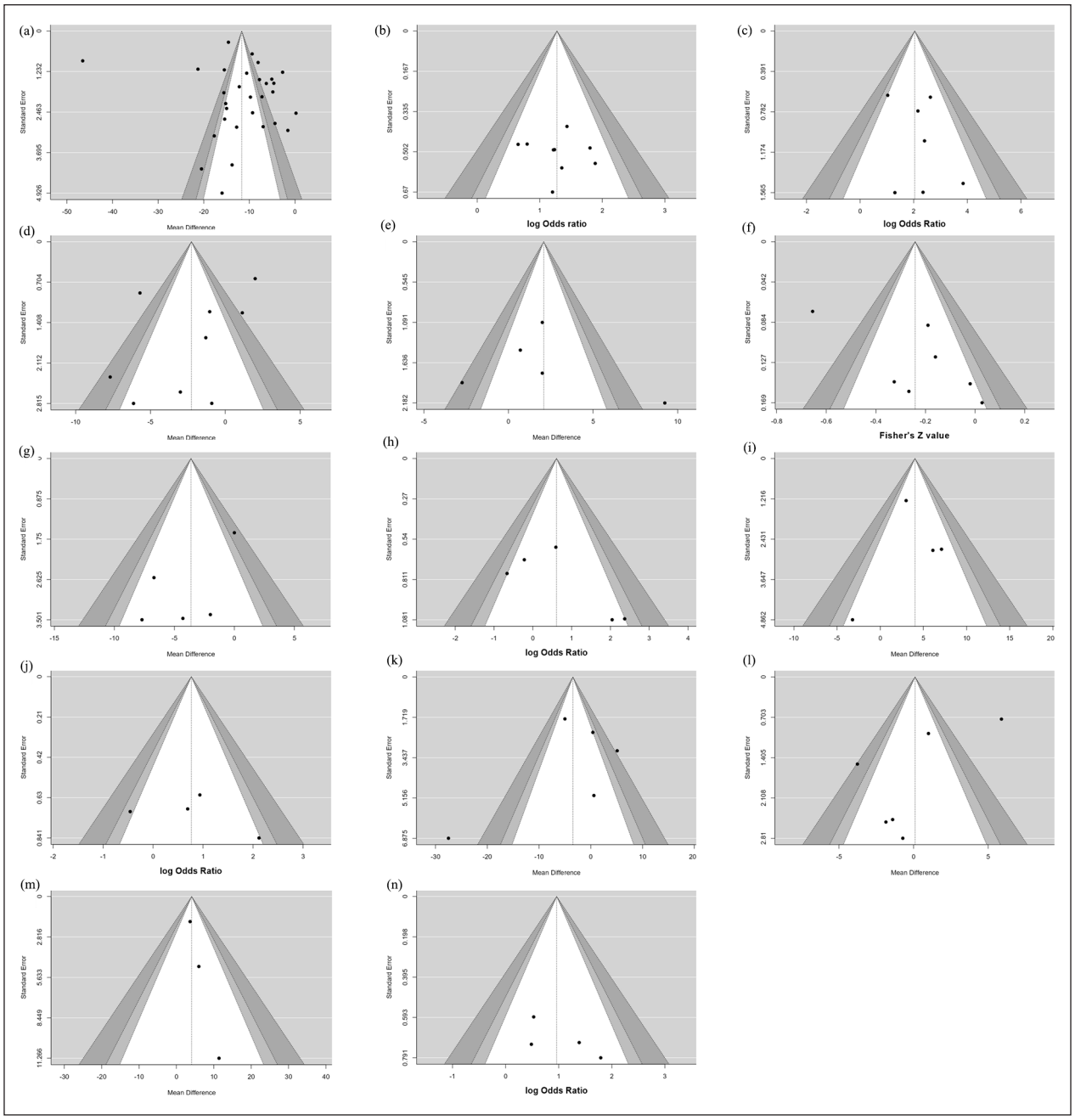

Figure 20. Funnel plots assessing publication bias: overall symmetrical funnel plots with no evidence of publication biases. (a) Vit D level in SSc patients vs. controls. (b) Vit D insufficiency in SSc patients vs. controls. (c) Vit D deficiency in SSc patients vs. controls. (d) Vit D level in dSSc vs.lSSc. (e) Vit D deficiency association with mRSS. (f) Correlation between Vit D level and mRSS. (g) Vit D level in patients with or without ILD. (h) Vit D deficiency association with ILD. (i) Vit D deficiency association with sPAP. (j) Vit D deficiency association with digital ulcers. (k) Vit D level in patients with or without digital ulcers. (l) Vit D level association with digestive tract involvement. (m) Vit D level in patients with or without calcinosis. (n) Association of Vit D insufficiency with anti-Scl70 Ab.

peculiar finding indicated that the decrease in Vit D level in dSSc versus lSSc was greater in female patients and in those with ILD. However, this nearly significant decrease of Vit D level in lSSc requires replication in further studies on independent cohorts.

In the current study, increase of mRSS in the case of Vit D deficiency failed to reach statistical significance (mean difference = 2.08 [−0.92 to 5.08], $p = 0.175$). However, there was a high level of between-studies heterogeneity, and the effect size could vary in 95% of comparable populations between −0.92 decrease and 5.08 increase in mRSS. Subgroup by ethnicity analysis showed stable association values. Conversely, patients' age was significantly correlated with the effect size, which indicated that the increase

of mRSS was rather observed in older patients. Besides, there was a significant correlation between Vit D level and mRSS (Pearson $r=-0.26$ [−0.44 to −0.08], $p=0.004$), though with a significant level of heterogeneity ($I^2=77\%$). After removing the Corrado 2015 study,[24] which was an outlying study and the source of all between-studies heterogeneity, the correlation between Vit D level and mRSS was moderate, Pearson $r=-0.19$ [−0.28 to −0.1]. As for the association between Vit D deficiency and mRSS, the correlation between Vit D level and mRSS was stronger in older patients. Besides, the significant negative correlation between Vit D level and mRSS points to a linear relationship, which might explain why the association of Vit D deficiency with mRSS did not reach the statistical significance. Indeed, subgrouping via transforming a continuous variable (Vit D level) into a categorical one (Vit D deficiency) can lead to a loss of statistical power.[62]

In the present meta-analysis, decreased Vit D level was significantly associated with ILD occurrence (mean difference=−3.61 [−6.93 to −0.3] ng/mL, $p=0.03$), though with a moderate amount of between-studies heterogeneity ($I^2=47\%$). Therefore, the effect size in 95% of comparable populations could fall between −13.88 ng/mL decrease and 6.66 ng/mL increase in Vit D level. Conversely, the risk of ILD conferred by Vit D deficiency was not statistically significant, (OR 95% CI=1.84 [0.66–5.14], $p=0.24$), though with a significant between-studies heterogeneity ($I^2=53\%$). Once again, the absence of statistical significance could have resulted from a loss of statistical power.[62] In order to explore the sources of the between-studies heterogeneity for the associations of Vit D level and Vit D deficiency with ILD occurrence, subgroup by ethnicity analyses and meta-regressions were performed. However, none of the examined covariates could explain the between-studies inconsistency. Other potential factors, such as the season of sampling, could have impacted these associations.

In this study, Vit D deficiency was found to be associated with increased sPAP, mean difference=4.17 [1.44–6.89] mmHg, $p=0.003$. Besides, there was a low level of heterogeneity ($I^2=20\%$), and the increase in sPAP could vary between 0.13 and 8.21 mmHg in 95% of comparable populations. Investigation of potential sources of heterogeneity showed a significant positive correlation between the effect size and patients' age. Hence, in the case of Vit D deficiency the increase in sPAP was larger in older SSc patients.

Overall, Vit D deficiency and Vit D level were not associated with DU occurrence. However, there were significant between-studies heterogeneities of $I^2=47\%$ and $I^2=83\%$, respectively. Subgroup analyses revealed significant inter-ethnic differences. Indeed, Vit D deficiency conferred an approximately 8.33-fold increased DU risk in Asians, and Vit D decrease was about –27.5 ng/mL in the case of DU occurrence. Conversely, no association was noted in Caucasians. These inter-ethnic disparities could be due to different genetic and environmental factors that might influence DU occurrence. In this regard, Vit D deficiency and/or Vit D level associations with DU were found to be positively correlated with patients' age, gender-ratio (M/F), and dSSc/lSSc ratio. These peculiar findings indicated that the association between Vit D and DU occurrence was rather noted in older cases, in male patients, and in dSSc subset. Nevertheless, as there were only five included studies for each comparison, further studies are needed in the future to better estimate Vit D role in DU occurrence.

Combined analysis showed no significant association between Vit D level and digestive tract involvement ($p=0.962$), though with a high level of between-studies heterogeneity ($I^2=70\%$). Exploring potential sources of heterogeneity through subgroup analyses and meta-regressions did not reveal any significant difference or correlation. Other factors such as disease activity and severity, which might vary between included studies, could explain the between-studies inconsistency. In this regard, more studies specifying disease activity and severity are required in the future to further determine the role of Vit D in DTI.

The present meta-analysis showed that vitamin D levels were significantly higher in patients with calcinosis than in those without, mean difference=4.18 [1.07–7.28], $p=0.008$. Besides, there was no between-studies heterogeneity ($I^2=0\%$), and meta-regression revealed no correlation of the effect size with patients' age and gender-ratio (M/F). Of note, there was only three included studies for the association of calcinosis with Vit D level. Hence, further studies are needed to confirm this association.

This study showed that Vit D insufficiency conferred a roughly 2.62-fold increase in Scl70 Ab occurrence. Although there was no between-studies inconsistency ($I^2=0\%$), meta-regression was performed and revealed a significant positive correlation of the effect size with patients' age. This finding suggested that the association between Vit D and Scl70 Ab occurrence was rather observed in older patients. Since Vit D exerts immunomodulatory effects and is essential for Treg function,[7–9] Vit D insufficiency/deficiency may promote the synthesis of autoantibodies in various autoimmune diseases, which could explain the observed association with anti-Scl70 antibodies. Besides, lower Vit D level might lead to a more diffuse SSc, which is characterized by the presence of anti-Scl70 Ab rather than ACA.

Combined analysis of only two included studies showed that Vit D deficiency was associated with a significant decrease in forced vital capacity, mean difference=−6.02 [−11.9 to −0.15], $p=0.04$. Although there was no between-studies inconsistency ($I^2=0\%$), we must acknowledge that this result, which was based on only two included studies, might lack precision and require replication in future studies.

In summary, the present meta-analysis revealed that decrease in Vit D was found to be associated with

susceptibility to SSc, increased mRSS, ILD occurrence, increased sPAP, decreased FVC, and Scl70 Ab presence. Conversely, calcinosis was associated with a higher level of Vit D. Nevertheless, there was significant between-studies heterogeneity for some comparisons. To the best of our knowledge, this study was the first to perform several subgroup analyses and meta-regressions in order to explore the aforementioned heterogeneity. Meta-regression provided the study with some interesting findings such as significant correlations with patient's age, gender-ratio (M/F), and dSSc/lSSc ratio. However, there are some limitations that need to be acknowledged. First, like other autoimmune diseases, SSc risk depends on several factors such as environmental, infectious, psychological and genetic, and as the present study analyses derived from pooling Vit D level and/or Vit D insufficiency/deficiency aggregate findings without any access to raw data, there was a lack of further adjustment for baseline characteristics. Second, the majority of included studies were performed in Caucasians and Asians, and few in Middle-Eastern populations and South Americans. Hence, the present meta-analysis results cannot be generalized to sub-Saharan African populations. Third, some investigated comparisons included only a small number of studies, and require therefore replications in independent large cohorts. Fourth, only two studies reported data on Valentini disease activity index and Medsger severity scale. This issue prevented us from adjusting the results on these two important factors that might impact all investigated comparisons.

Conclusion

This meta-analysis showed that decrease in Vit D was found to be associated with susceptibility to SSc, increased mRSS, ILD occurrence, increased sPAP, decreased FVC, and Scl70 Ab presence. Conversely, calcinosis was associated with a higher level of Vit D.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Supplemental material

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